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SOMATOTROPHIC EFFECT OF PITUITARY GRAFTS AND TUMORS

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SOMATOTROPHIC EFFECT OF PITUITARY GRAFTS AND TUMORS

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A Thesis Presented to the Faculty of the
Yale University School of Medicine
in Partial Fulfillment of the Requirements
for the Degree

Doctor of Medicine

Department of Anatomy
Yale University School of Medicine

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INTRODUCTION

Studies on the hypothalamic control of endocrine secretions have tended to focus primarily on the gonadotrophic, adrenocorticotrophic, and thyrotrophic functions of the pituitary gland (1). In each case a target organ is available with which to monitor changes of these functions and hence to study their possible mediation by the central nervous system. Somatotrophin (STH) influences no specific local target organ and perhaps for this reason the role of the hypothalamus in regulating STH production has been somewhat neglected.

Although interest in hypothalamic control of somatotrophin has developed relatively recently, observations indicating a link between the central nervous system and growth can be found in the early experiments of Ranson and co-workers on hypothalamic obesity. In 1942 Hetherington and Ranson (2) reported that some of their rats with bilateral hypothalamic lesions showed evidence of growth retardation in terms of decreased nose-anus length even though they appeared markedly obese. Bogdanove and Lipner (3) found that some of their rats with lesion-induced obesity gained less weight than controls and that the pituitary glands in these animals showed striking degranulation of acidophils.

Growth retardation following hypothalamic lesions was subsequently confirmed by Endröczi, et al. (4), Hinton and Stevenson (5), Reichlin (6,7), and Marescotti, et al. (8). Hinton and Stevenson placed bilateral lesions in the anterior

hypothalamus of male rats and noted that although somatic growth was reduced, thyroids, adrenals, and gonads in these animals appeared normal at autopsy. Reichlin (6,7) found that no specific localization within the hypothalamus could be made of a lesion responsible for poor growth although the most striking effects were seen in rats with large lesions involving the median eminence, portal plexus, and infundibular stalk. It appeared initially that the effects on growth might be explained on the basis of damage to the blood supply of the anterior pituitary rather than on a particular neural defect. However, india ink perfusion studies suggested that the pituitary gland remained well vascularized after hypothalamic lesions. In addition, growth was retarded in lesioned animals despite maintenance therapy with thyroid hormone and gonadal steroids and even with the precaution of paired feeding.

Spirtos and Halmi (9), concerned with the diabetogenic action of growth hormone, found that rats with hypothalamic lesions showed an exaggerated fall in blood sugar following insulin injections. The increased insulin sensitivity was due, they postulated, to a decrease in somatotrophin because all of the animals treated with STH became more insulin resistant whereas only 50% of those given cortisone responded in this way. Harris (10) had previously demonstrated a marked decrease in insulin sensitivity following prolonged electrical stimulation of the tuberal region in rabbits. No studies were done to determine whether the alteration in insulin sensitivity was due to increased STH, increased ACTH, or

merely resulted from stress-induced liberation of epinephrine and secondary hyperglycemia.

With the evidence presented implicating the hypothalamus as a regulator of growth hormone secretion, several laboratories attempted to demonstrate autonomous production of growth hormone by pituitary grafts. Martini, et al. (11) and Greer (12) transplanted pituitary glands to hypophysectomized rats and found that the growth of grafted hosts was no greater than that of non-grafted controls. Hertz (13), however, obtained suboptimal growth at two thirds the normal rate by transplanting four pituitary glands beneath the kidney capsule in weanling hypophysectomized rats. He concluded that in the rat STH production is not completely dependent upon an intact hypothalamo-hypophyseal system.

Thompson, et al. (14) working with an in vitro system suggested that growth hormone may be produced by heterogeneous cell cultures of human adenohypophysis. STH was assayed using the tibia method of Evans (15) in which the test substance is injected into immature hypophysectomized female rats and after four days the width of the proximal epiphyseal cartilage plate of the tibia is measured. Although a number of hormones will increase the width of the cartilage plate, it responds with greatest sensitivity to growth hormone and only with growth hormone is a proportionality obtained between dose and response (16).

Using the tibia test as an assay for growth hormone Reichlin (17) obtained direct evidence that some phase of STH

secretion is affected by hypothalamic injury. In rats with lesions in the anterior hypothalamus and median eminence, a decrease in growth rate was accompanied by significant reduction in the growth hormone content of pituitary glands. Evidence suggesting the presence of a somatotrophin-releasing factor in hypothalamic extracts has recently been presented by Franz, et al. (18). These investigators found that acetic acid extracts of hog hypothalami produced widening of the tibial cartilage plate in intact but not hypophysectomized immature female rats. This activity was not present in extracts prepared from regions of brain other than hypothalamus.

A recent publication adds clinical evidence to the experimental data that has accumulated on the relationship between the hypothalamus and growth hormone secretion. Leszynsky (19) reported a fascinating case of classic acromegaly developing in a young woman during pregnancy and progressing after delivery. Additional clinical features included persistant lactation and latent diabetes mellitus. This patient has been observed carefully for a period of four years with no sign of a gross pituitary lesion. Although no definitive diagnosis has been established Leszynsky considers this to be a case of hypothalamic acromegaly.

The present investigation was undertaken to explore further the autonomy of pituitary growth hormone secretion. By transplanting pituitary glands directly to the region of the proximal tibial epiphysis it was hoped that the continuous

secretion of small amounts of growth hormone would be sensitively recorded by an increase in the width of the cartilage plate. By doing unilateral transplantations in normal and hypophysectomized hosts each animal would serve as its own control. Should locally produced growth hormone gain access to the vascular system and exert a systemic effect, then comparisons could be made between animals bearing pituitary grafts and untreated controls.

MATERIALS AND METHODS

Part I. Somatotrophic effect of pituitary grafts.

Forty mice of the CC hybrid stock (CBA x C_{57}) were divided into the following groups (Table 1):

1) Untreated controls.

2) Hypophysectomized with two pituitary glands transplanted to left tibia (to be referred to as hypophysectomized mice).

3) Two pituitary glands transplanted to left tibia (to be referred to as pituitary-transplanted mice).

4) Treated with growth hormone.

Hypophysectomy was performed when the mice were 26-28 days of age under amobarbital anesthesia using the method of Thomas (20,21). Pituitary glands were transplanted when the hosts were 32-33 days old with donors selected from adult mice of the same sex and stock. Mice of the fourth experimental group received daily intraperitoneal injections of 1.0 mg. bovine growth hormone[1] starting at 33 days of age. Water and Purina Laboratory Chow were available ad libitum. The regimen for hypophysectomized mice was supplemented by adding 2% dextrose in 0.9% NaCl to the drinking water. Autopsies were performed on all animals at 51-53 days of age; that is, three weeks after transplantation or the onset of growth hormone injections.

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1. The bovine growth hormone used was the preparation NIH-GH, B-2 distributed by the Endocrinology Study Section, National Institutes of Health.

Table 1. Composition of experimental groups and age in days at time of hypophysectomy, transplantation, hormone treatment, and autopsy.

Group	n Sex	Age at hypophysectomy	Age at transplantation or hormone treatment	Age at autopsy
Control	8 3M 5F			51-52
Hypox	7 2M 5F	27	32	52
Incomplete hypox	5 2M 3F	26-28	32-33	51-53
Pituitary transplanted	10 4M 6F		32-33	52
Growth hormone-treated	10 5M 5F		33	51-52

Pituitary glands were transplanted by making a one centimeter incision on the lateral aspect of the left leg, retracting the tibialis anterior muscle laterally near the proximal tibia, and placing the graft between muscle and bone. Often a small portion of muscle was excised to create room for the graft. The tibialis anterior fascia was sutured to the crural fascia over the tibia in order to prevent the soft pituitary tissue from exuding into the subcutaneous space. Placement was intended to insure relatively close contact of donor pituitary tissue and host epiphyseal cartilage plate. A similar procedure was carried out on the contralateral leg except that no graft was inserted because some alteration in the architecture of the epiphysis might be attributed to surgical trauma and secondary vascular changes. Slight transient impairment of dorsiflexion was frequently noted postoperatively.

At autopsy the sella turcica was examined carefully under a dissecting microscope for residual pituitary tissue; the thymus, adrenals, gonads, and accessory reproductive structures were dissected out, weighed, and fixed in Bouin's solution, and the pituitary graft was recovered and fixed in Bouin's. The remaining viscera were removed leaving only the musculoskeletal frame which was pinned flat on a cork board and fixed in 10% formalin. Roentgenograms were made of the skeletal system of each animal and measurements were taken from the films of the long bones and the lumbar, sacral, and first six caudal vertebrae. All roentgenograms were taken

with the same machine, the skeleton was placed directly on the film cassette, and a uniform tube-to-object distance was used throughout the study.

The tibias were then dissected out and decalcified by immersion in DeCal[1] for 24 hours. They were rinsed for three hours in running water, split longitudinally with a razor blade, and embedded in paraffin. Sections were cut at 7 microns and stained with hematoxylin and eosin. The proximal epiphyseal cartilage plate was evaluated by the growth hormone bio-assay method of Evans (15). The epiphyseal plate was measured with an ocular micrometer at 100x magnification with one ocular unit equal to 8.9 microns. Ten measurements were taken for each tibial epiphyseal plate going from the anterior to posterior surface, and the mean value was used in subsequent calculations.

Representative pituitary grafts recovered from hypophysectomized and transplanted animals were studied histologically for the presence of viable tissue.

All animals were weighed daily during the period of observation.

Part II. Somatotrophic effect of pituitary tumors.

A second goup of mice bearing subcutaneous transplants of a pituitary tumor was also studied. These were mice of the CB stock (C_{57} x BC) carrying the fourth transfer generation of a transplanted pituitary tumor designated 59 CB_2D.

1. Scientific Products, Long Island, New York.

The animals were one to two years old and had received transplants of the tumor at two to four months of age. The tumor was being followed in the laboratory for possible hormone dependency and production. Some of the tumor-bearing animals had abnormally high body weights (without excessive fat deposition or apparent edema) and marked splanchnomegaly; thus it appeared that the tumor transplant might be secreting growth hormone.

Twenty-three animals from this group were studied. Six were excluded because they had been previously castrated or had received pellets of stilbestrol-cholesterol which might be expected to influence skeletal growth. This report includes data from the remaining 17 mice, 8 of which had prominent tumor masses at autopsy and 9 in which little or no viable tumor could be found.

Five mice of the compatible CB_1A stock were hypophysectomized at 27 days of age and at 33 days portions of tumor tissue roughly equivalent to two pituitary glands were transplanted to the proximal left tibia as in Part I. Autopsies were performed on these animals two weeks after transplantation.

All animals in the tumor group were studied in the same manner as in Part I except that weights of liver, spleen, and kidneys were also obtained and representative mammary glands were saved for whole-mount preparations.

In evaluating data from Parts I and II the significance of difference between means was tested using Student's t test.

The following formulas were used:

$$\text{Standard Error} = \sqrt{\frac{\Sigma (x-m)^2}{n(n-1)}}$$

$$t = \frac{m_1 - m_2}{\sqrt{(\text{S.E. } m_1)^2 + (\text{S.E. } m_2)^2}}$$

$$\text{Degrees of Freedom} = n - 1 \quad .$$

OBSERVATIONS

PART I

BODY WEIGHT

All mice were comparable in weight at the start of the experiment, although daily observations were begun earlier in the hypophysectomized animals. Controls, pituitary-transplanted, and growth hormone-treated mice all showed a similar weight response; males gained at least six grams and females at least four grams (Figures 1 and 2). The pattern of weight gain was useful as an early index of completeness of hypophysectomy. After complete removal of the pituitary gland the mice continued to lose weight during the first week after surgery, then returned to pre-operative weight (at about the time of pituitary transplantation), and did not exceed this by more than one to two grams. Animals with a weight gain after surgery that exceeded pre-operative weight by more than two grams were subsequently found to fulfill the remaining three criteria established during the experiment for incomplete hypophysectomy: 1) pituitary fragments remained in the sella at autopsy, 2) the target organs failed to atrophy, and 3) the length of the lumbar vertebral segment was in the control range. Five out of twelve hypophysectomies were judged to be incomplete by these criteria. After an initial lag phase the weight curve for the incompletely hypophysectomized mice became similar to that of mice in the control group.

Fig. 1

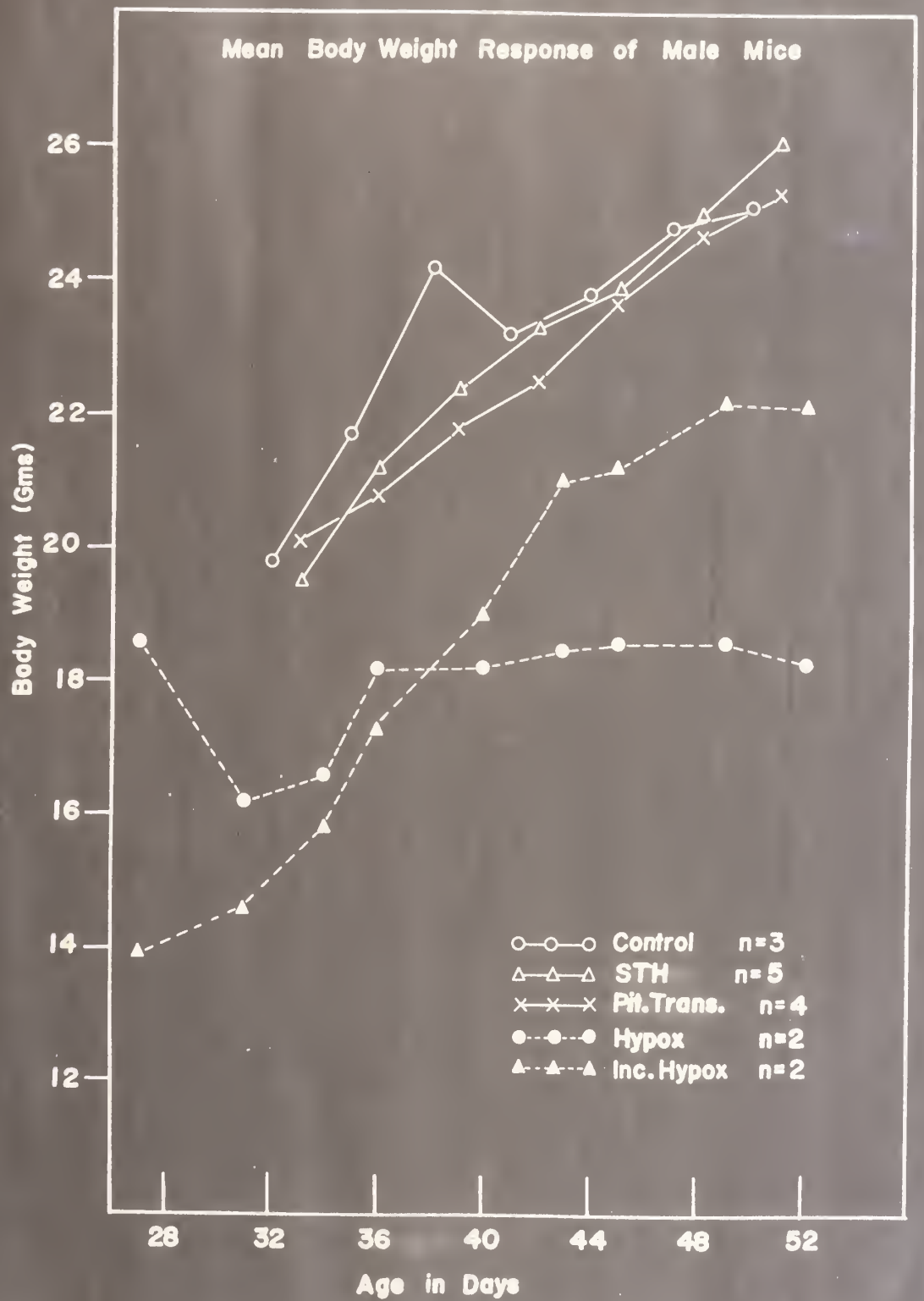
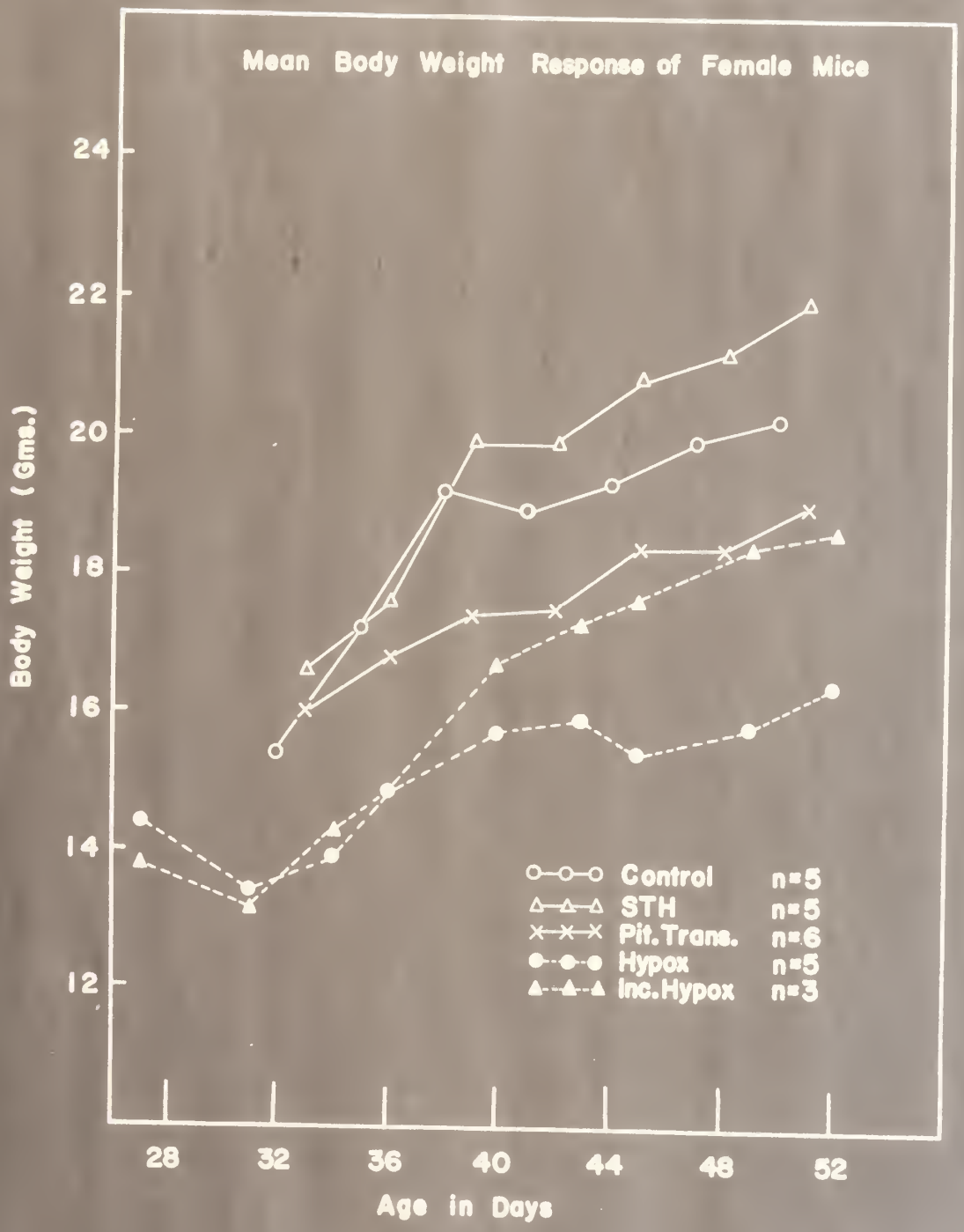

Mean Body Weight Response of Male Mice
Body Weight (Gms)
26
24
22
20
18
16
14
12
28
32
36
40
44
48
52
Age in Days
Control n=3
STH n=5
Pit.Trans. n=4
Hypox n=2
Inc. Hypox n=2

Fig. 2


Mean Body Weight Response of Female Mice
Body Weight (Gms.)
24
22
20
18
16
14
12
28
32
36
40
44
48
52
Age in Days
Control n=5
STH n=5
Pit. Trans. n=6
Hypox n=5
Inc. Hypox n=3

ORGAN WEIGHTS

Weights of adrenals, gonads, and accessory reproductive structures were reduced in all hypophysectomized animals (Table 2). Weight of the thymus in hypophysectomized males was more than double the weight in control mice. In all except hypophysectomized mice the thymic weight in males was approximately half that of the females; thymic weights in hypophysectomized animals were indentical for males and females. Organ weights in pituitary transplanted, growth hormone-treated, and incompletely hypophysectomized mice did not differ from controls.

SKELETAL SYSTEM

Measurements of the humerus, ulna, femur, and tibia were similar for mice of all groups although mean lengths for hypophysectomized animals were slightly less than those obtained for controls. In hypophysectomized, incompletely hypophysectomized, and pituitary-transplanted animals there was no difference in length between the left (experimental) and right (control) tibias (Table 3).

Although measurements of the appendicular skeleton of mice in the different experimenta, groups were similar, the axial skeleton proved highly sensitive to alterations in the level of growth hormone. Mean length of the lumbar vertebral segment (six lumbar vertebrae including five intervertebral discs) was 18.3 mm. for controls as compared to 15.7 mm. for hypophysectomized mice ($P<.0005$) and 19.3 mm. for mice treated

Table 2. Mean weight and range in mg. of thymus, adrenals, gonads, and accessory reproductive structures.

Group	Control	Hypox	Incomplete hypox	Pituitary transplanted	Growth hormone-treated
n M	3	2	2	4	5
Thymus	35.9 (31.2-42.8)	73.0 (71.4-74.6)	44.3 (43.2-45.4)	34.4 (24.2-45.8)	44.8 (41.8-48.2)
Adrenals	4.3 (4.0-4.8)	1.6 (1.6)	3.8 (3.8)	4.4 (4.0-4.8)	4.4 (3.6-4.8)
Testes	184.4 (160.4-211.0)	36.2 (32.4-40.0)	168.1 (166.6-169.6)	195.6 (188.0-205.2)	197.9 (182.6-214.2)
Seminal vesicles & prostate	142.6 (128.2-152.2)	8.2 (8.2)	139.5 (137.6-141.4)	166.1 (104.2-210.0)	131.5 (125.0-146.0)
n F	5	5	3	6	5
Thymus	72.0 (66.2-78.0)	72.9 (59.2-84.6)	70.5 (67.6-75.6)	64.1 (50.0-74.2)	61.4 (50.4-68.4)
Adrenals	5.4 (5.0-5.8)	2.6 (2.0-3.0)	4.7 (3.8-5.6)	5.1 (4.8-5.4)	5.5 (4.8-6.4)
Ovaries	5.4 (4.0-6.0)	1.4 (0.8-1.8)	4.4 (2.2-5.6)	5.3 (3.2-7.8)	5.4 (3.2-7.8)
Utero-cervix	59.3 (42.6-94.4)	8.1 (7.2-8.8)	86.5 (16.8-187.8)	74.2 (13.4-116.6)	55.0 (26.4-84.2)

Table 3. Measurements of long bones. Mean length and range in mm. of left humerus, ulna, and femur, and left and right tibias.

Group	n Sex	Left humerus	Left ulna	Left femur	Tibia Left	Tibia Right
Control	8 3M 5F	10.8 (10.1-11.6)	13.0 (12.6-14.0)	14.2 (13.8-14.8)	16.6 (16.2-17.3)	16.8 (16.4-17.4)
Hypox	7 2M 5F	10.3 (9.9-10.6)	12.5 (12.0-13.3)	13.2 (12.8-13.5)	15.9 (15.6-16.2)	15.8 (15.5-16.3)
Incomplete hypox	5 2M 3F	10.2 (10.0-11.5)	12.8 (12.5-13.0)	13.8 (13.2-14.5)	16.2 (15.6-16.5)	16.3 (15.9-16.7)
Pituitary transplanted	10 4M 6F	10.9 (10.0-11.5)	13.2 (12.6-14.4)	14.1 (13.0-15.1)	16.6 (15.8-17.2)	16.7 (15.8-17.4)
Growth hormone treated	10 5M 5F	11.1 (10.4-11.5)	13.4 (12.7-13.9)	14.1 (13.7-14.9)	16.7 (16.4-17.1)	16.9 (16.5-17.4)

with growth hormone ($P < .01$). Pituitary-transplanted and incompletely hypophysectomized animals had mean lumbar vertebral lengths that did not differ significantly from controls (Table 4 and Figures 3, 4, and 5).

Measurements of the sacral and first six caudal vertebrae showed mean lengths for hypophysectomized animals less than controls and mean lengths for growth-hormone treated animals slightly increased. The differences, although statistically significant, were less striking than those for the lumbar vertebral segment.

WIDTH OF PROXIMAL TIBIAL EPIPHYSIS

Widths of the proximal epiphyseal cartilage plates were obtained for the left tibia in untreated and growth hormone-treated animals and for both the left (experimental) and right (control) tibias in mice that had received pituitary grafts. The "n" values for the left and right tibias in hypophysectomized and pituitary-transplanted animals differ because a few cartilage plates were inadvertently removed during dissection of the tibias.

All mice with pituitary transplants had significantly wider left tibial cartilage plates than the controls; the contralateral epiphysis (sham operated) did not differ in width from the control value, but was significantly narrower than the left epiphysis. In growth hormone-treated animals the left tibial cartilage plate was also significantly wider than in controls (Table 5, Figure 6).

Table 4. Measurements of axial skeleton. Mean length in mm. ± standard error of lumbar, sacral, and first six caudal vertebrae.

Group	Control	Hypox	Incomplete hypox	Pituitary trans-planted	Growth hormone treated
n Sex	8 3M 5F	7 2M 5F	5 2M 3F	10 4M 6F	10 5M 5F
Lumbar	18.3 ± 0.26	15.7 ± 0.17	17.6 ± 0.29	18.1 ± 0.30	19.3 ± 0.17
Significance compared to control value	-	$P<.0005$	n.s.[1]	n.s.	$P<.01$
Sacral	16.5 ± 0.16	15.2 ± 0.21	15.8 ± 0.31	16.3 ± 0.23	17.1 ± 0.21
Significance compared to control value	-	$P<.005$	n.s.	n.s.	$P<.05$
Caudal	23.7 ± 0.26	22.5 ± 0.24	22.6 ± 0.46	23.1 ± 0.22	24.7 ± 0.33
Significance compared to control value	-	$P<.01$	n.s.	n.s.	$P<.025$

[1] n.s. (not significant) = $P>.05$.

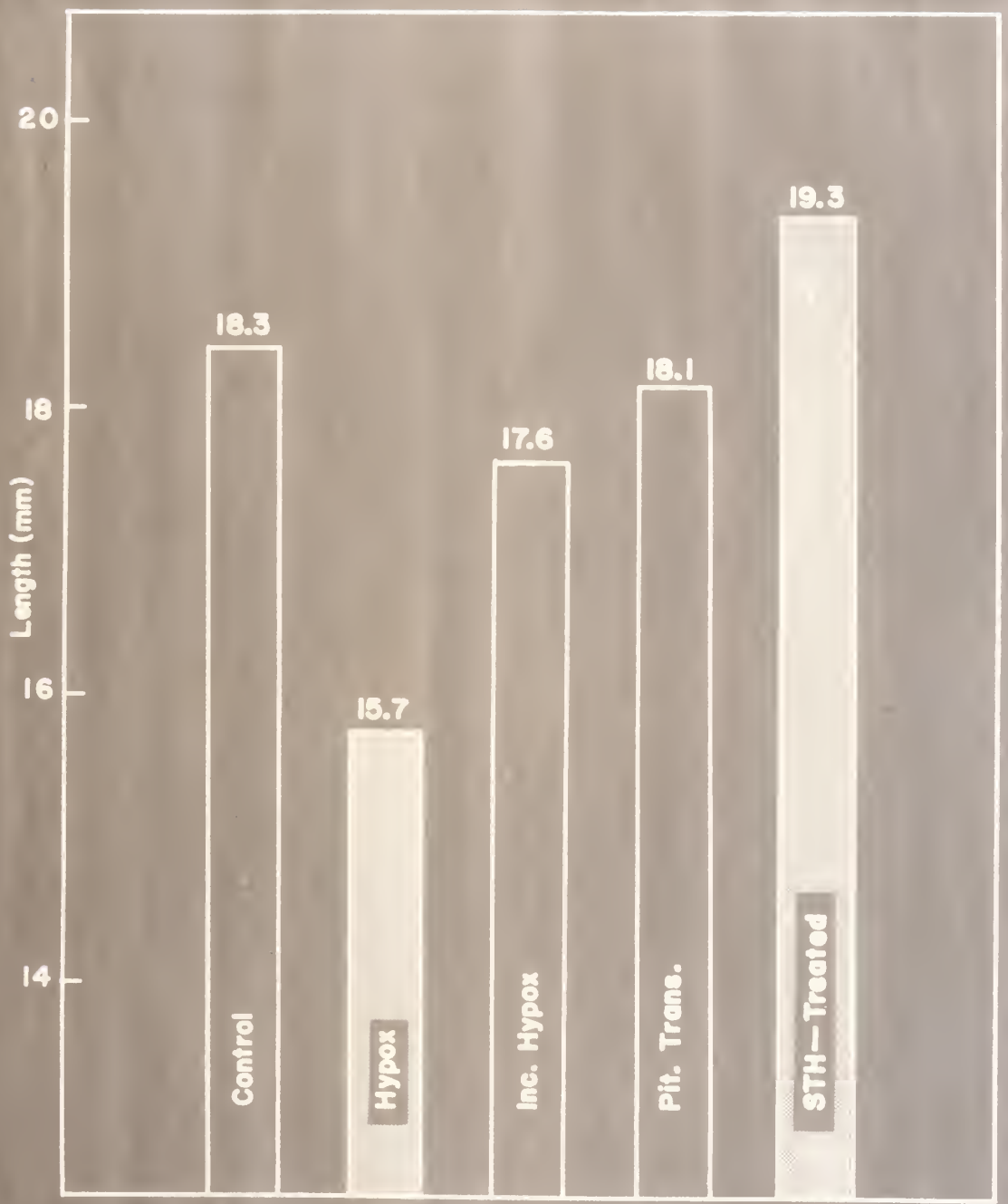


Fig. 3

Mean Length of Lumbar Vertebral Segment in mm.

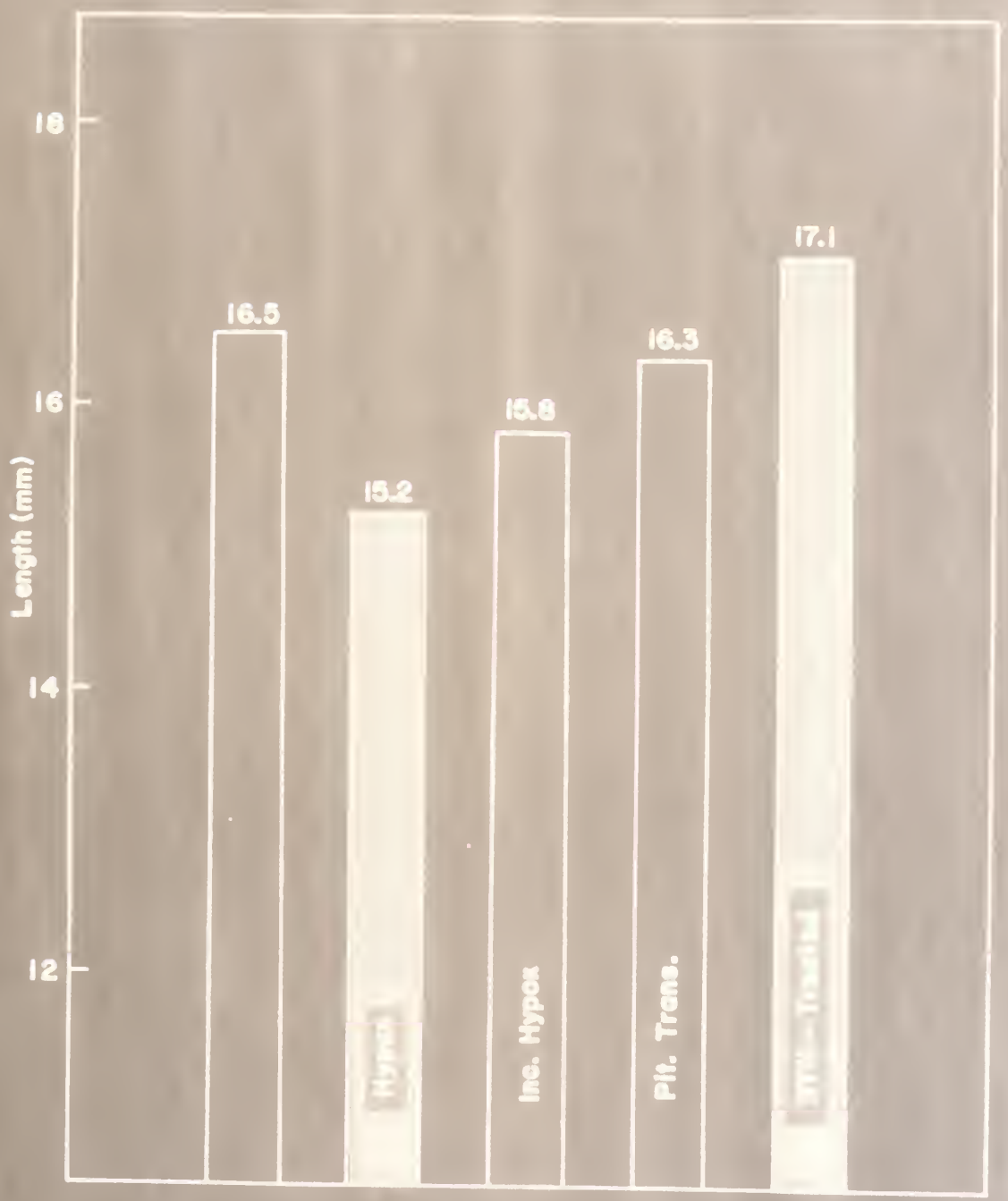


Fig. 4

Mean Length of Sacral Vertebral Segment in mm.

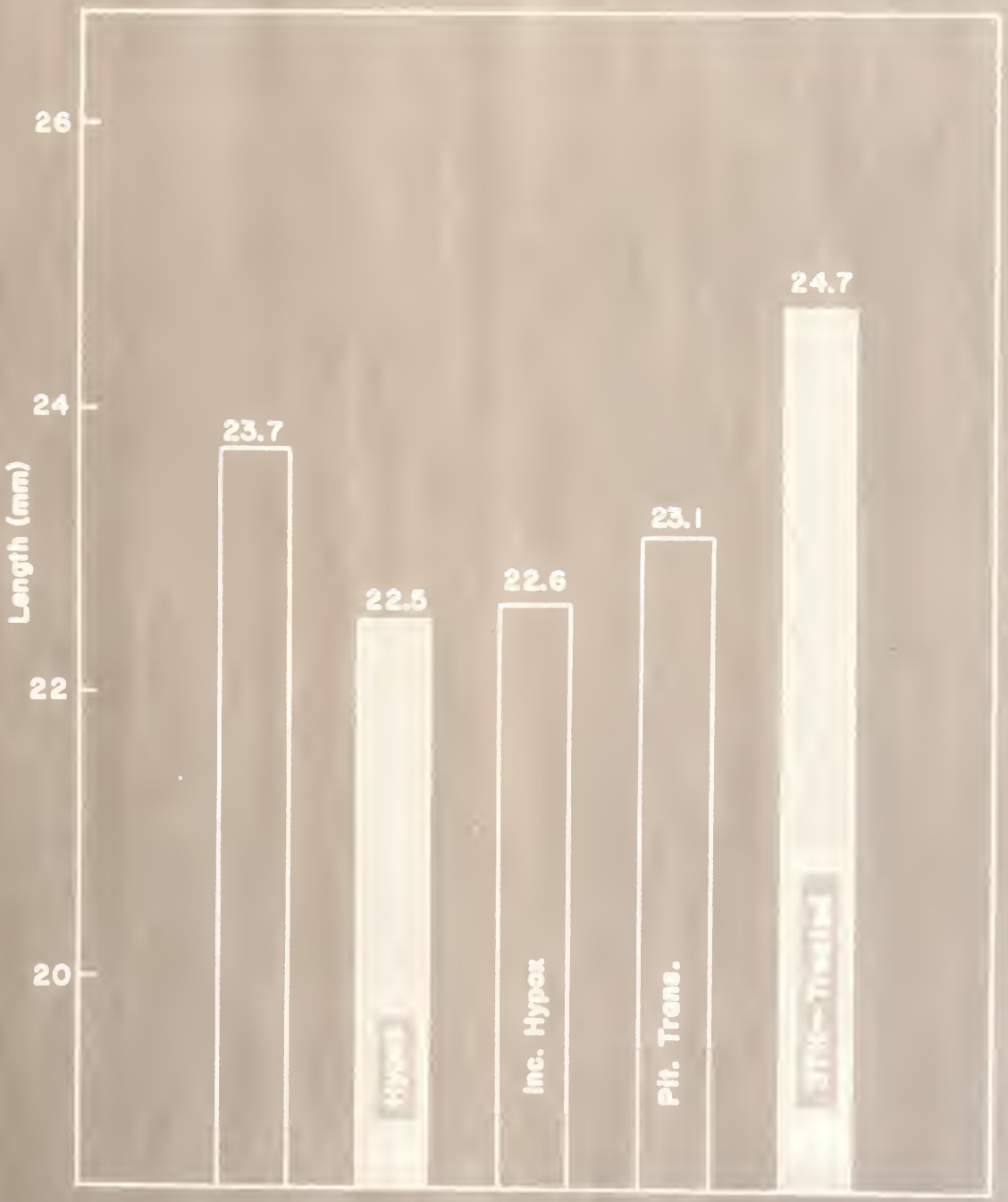


Fig. 5

Mean Length of First Six Caudal Vertebrae in mm.

Table 5. Measurements of tibial epiphysis. Mean width of cartilage plate in microns ± standard error.

Group	n Sex	Epiphyseal width in microns	Significance compared to control value	Significance difference between L. and R.
Control	7			
L. Tibia	3M 4F	254.7 ± 6.44	-	-
Hypox	6			
L. Tibia	2M 4F	320.9 ± 6.93	P<.0005	P<.005
R. Tibia	7			
	2M 5F	267.6 ± 5.26	P>.05	-
Incomplete hypox	5			
L. Tibia	2M 3F	301.5 ± 5.44	P<.005	P<.005
R. Tibia	5			
	2M 3F	260.4 ± 5.40	P>.25	
Pituitary transplanted	10			
L. Tibia	4M 6F	289.6 ± 5.24	P<.005	P<.005
R. Tibia	9			
	4M 5F	255.1 ± 4.62	P>.25	
Growth hormone treated	9			
L. Tibia	4M 5F	295.6 ± 2.34	P<.005	-

Fig. 6

Mean Width of Tibial Epiphyseal Cartilage Plate in μ.

PART II

Autopsies were performed on mice in the tumor-bearing group at random intervals over a two-month period. Animals were assigned to the experimental or control groups on the basis of tumor size, with 5 x 5 mm. arbitrarily selected as the upper limit for the control group.

The mean age at the time of tumor transplantation was 91 days for control and 82 days for experimental animals (Table 6). It took an average of 302 days from the time of transplantation until the tumor was clinically apparent (1 x 1 mm., usually) in the control group as compared to 141 days for the experimental group. The four tumors in mice of the control group ranged in size from 1 x 1 mm. to 4 x 3 mm. and were present (apparent to palpation) an average of 199 days prior to autopsy. The 8 tumors in mice of the experimental group ranged from 14 x 19 mm. to 22 x 25 mm. and were present an average of 212 days prior to autopsy. The mean age at autopsy was 603 days for controls and 434 days for experimentals.

BODY WEIGHT

The mean body weight for experimental animals with large tumors was 45.9 gm. as compared to 30.1 gm. for controls ($P<.0005$).

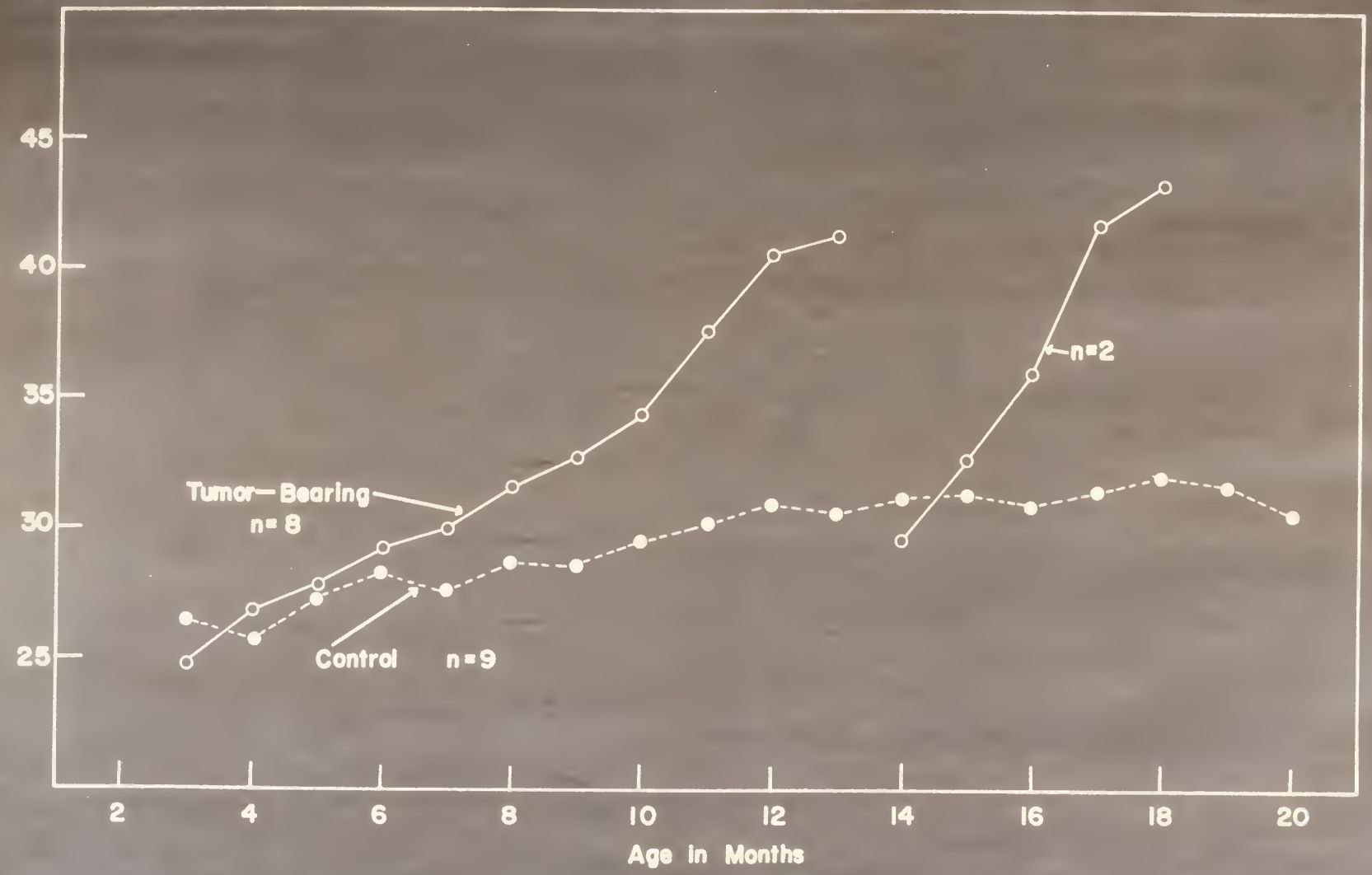
Changes in mean body weight for mice of both groups plotted at monthly intervals during the first 20 months of observation are shown in Figure 7. The curve for the experimental mice rises sharply from 6 to 13 months (29.2 gms. to

Table 6. Summary of age in days at time of tumor transplantation, number of days until appearance of tumor, duration of tumor presence and age at autopsy in control and experimental animals.

No. and sex	Age at transplantation	Days until appearance of tumor	Days tumor present	Age at autopsy
EXPERIMENTAL				
39M	69	134	209	412
58M	73	140	203	416
40M	69	168	175	412
27F	102	182	246	530
44F	78	134	174	386
43F	78	77	231	386
45F	78	134	174	386
18F	109	156	280	545
Mean	82.0	140.6	211.5	434.1
Range	69-109	77-280	174-280	386-545
CONTROL				
1M	119	—	-	730
21M	63	288	284	635
20M	63	331	241	635
37M	122	—	-	565
42M	69	—	-	511
46F	78	266	42	386
13F	117	323	230	670
15F	77	-	-	629
15F	114	-	-	667
Mean	91.3	302	199.2	603.1
Range	63-122	266-331	42-284	386-730

Fig. 7

Mean Body Weight Response of Tumor—Bearing and Control Mice



41.3 gms.) whereas the control curve remains in the 28 to 32 gm. range. There is a second rise in the experimental curve beginning at 14 months. The points on this portion of the curve represent means for two animals with tumors that did not become apparent until nine months; hence these animals were "out of phase" with the rest of the group.

ORGAN WEIGHTS

Mean organ weights were calculated on the basis of relative rather than absolute weight (mg. organ weight / gm. body weight). Relative weights of liver, spleen, and kidneys were significantly greater in experimental animals with values for liver and spleen almost double the control weights (Tables 7, 8, and 9). Relative weights of thymus, adrenals, and gonads were similar in mice of both groups.

SKELETAL SYSTEM

Measurements of the long bones were comparable in the two groups whereas the axial skeleton showed marked differences (Tables 10 and 11). Mean lengths of the lumbar, sacral, and caudal vertebral segments in experimental mice were 26.1, 19.1, and 26.6 mm., respectively. Corresponding lengths in control mice were 21.4, 18.0, and 25.2 mm.; all differences were highly significant.

TIBIAL EPIPHYSEAL CARTILAGE

Sections through the proximal tibia in both control and experimental animals showed bony fusion of the epiphyseal

Table 7. Control group. Individual and mean body weight in gms. Individual and mean weights of liver, spleen, and kidneys in mgs. (absolute) and in mgs. per gm. of body weight. Dimensions of tumor at autopsy in mm.

No. and sex	Body weight gms.	Liver mgs.	Liver mgs./gm.	Spleen mgs.	Spleen mgs./gm.	Kidney mgs.	Kidney mgs./gm	Tumor size mm.
1M	25.2	2750[1]		39.7[1]		372.4	14.8	-
21M	32.4	1750	54.0	105.3	3.25	508.4	15.7	2 x 4
20M	35.0[2]							2 x 2
37M	37.7	1750	46.4	100.8	2.67	636.2	16.9	-
42M	32.3	1500	46.4	99.2	3.07	564.4	17.5	-
46F	28.4	1600	56.3	88.8	3.13	362.0	12.7	1 x 1
13F	28.8	1500	52.1	69.2	2.40	409.6	14.2	3 x 4
15F	25.8	1200	46.5	183.2[1]		374.0	14.5	-
16F	25.7	1400	54.5	89.2	3.47	377.2	14.7	-
Mean	30.1		50.9		3.00		15.1	

1. Animal developed lymphoma terminally involving liver and/or spleen; this weight therefore not used in calculating mean.

2. Animal died during night after weekly observation period. Sufficient post-mortem change had occurred to render organ weights and histology of little value.

Table 8. Experimental group. Individual and mean body weight in gms. Individual and mean weights of liver, spleen, and kidneys in mgs. (absolute) and in mgs. per gm. of body weight. Dimensions of tumor at autopsy in mm.

No. and sex	Body weight gms.	Liver mgs.	Liver mgs./gm.	Spleen mgs.	Spleen mgs./gm.	Kidney mgs.	Kidney mgs./gm.	Tumor size mm.
39M	50.1	5000	99.8	284.4	5.68	1050.0	21.0	14 x 23
58M	47.9	4500	93.9	230.4	4.81	956.7	20.0	22 x 25
40M	51.1	5000	97.8	290.0	5.68	1200.0	23.5	16 x 30
27F	46.0	4500	97.8	316.4	6.88	743.0	16.2	18 x 22
44F	42.3	3850	91.0	282.0	6.67	726.4	17.2	14 x 19
43FF	37.3	3250	87.1	194.0	5.20	620.2	16.6	13 x 21
45F	49.2	4750	96.5	355.4	7.22	792.0	16.1	20 x 22
18F	43.4	3500	80.6	330.5	7.62	718.8	16.6	16 x 25
Mean	45.9		93.1		6.22		18.4	

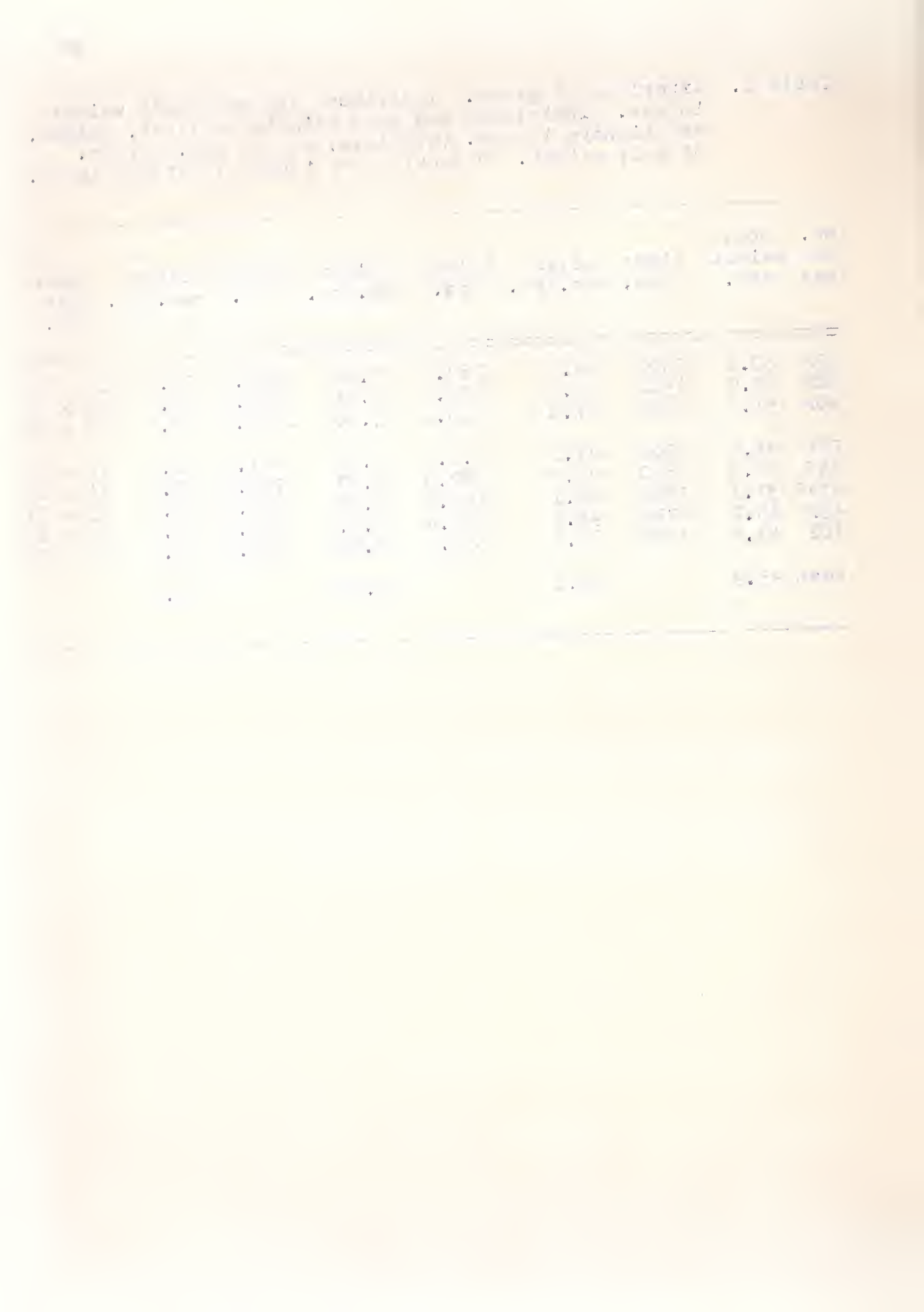


Table 9. Summary of mean body weight in gms. and mean weights of liver, spleen, and kidneys in mgs. per gm. body weight ± standard error.

Group	Control	Experimental
n	9	8
Sex	5M 4F	3M 5F
Body Weight (gms.)	30.1 ± 1.48	45.9 ± 1.65
Significance Control vs. Experimental	P<.0005	
Liver mgs. per gm. body weight	50.9 ± 1.64	93.1 ± 2.31
Significance Control vs. Experimental	P<.0005	
Spleen mgs. per gm. body weight	3.00 ± 0.16	6.22 ± 0.36
Significance Control vs. Experimental	P<.0005	
Kidney mgs. per gm. body weight	15.1 ± 0.54	18.4 ± 0.98
Significance Control vs. Experimental	P<.025	

Table 10. Measurements of long bones. Mean length and range in mm. of left humerus, ulna, femur, and tibia.

Group	n Sex	Left humerus	Left ulna	Left femur	Left tibia
Control	9 5M 4F	11.6 (10.6- 12.4)	13.3 (12.5- 13.8)	15.3 (15.0- 15.7)	17.7 (17.4- 18.0)
Experimental	8 3M 5F	12.5 (11.7- 13.5)	13.7 (13.3- 14.1)	15.2 (14.8- 15.7)	18.2 (17.4- 19.0)

Table 11. Measurements of axial skeleton. Mean length in mm. ± standard error of lumbar, sacral, and first six caudal vertebrae.

Group	Control	Experimental
n Sex	9 5M 4F	8 3M 4F
Lumbar	21.4 ± 0.07	26.1 ± 0.34
Significance Control vs. Experimental	-	P<.0005
Sacral	18.0 ± 0.14	19.1 ± 0.14
Significance Control vs. Experimental	-	P<.0005
Caudal	25.2 ± 0.23	26.6 ± 0.26
Significance Control vs. Experimental	-	P<.005

plate. This observation should be of help in deciding approximately when the tumor began to exert a somatotrophic effect; however, data concerning the normal age at which epiphyseal fusion occurs in mice could not be found. Because no evidence for somatotrophic stimulation could be found by histologic or roentgenographic study of the appendicular skeleton, it is probable that active hormone secretion by the tumor began after epiphyses had fused.

TUMOR TRANSPLANTS IN HYPOPHYSECTOMIZED MICE

In the hypophysectomized CB_1A mice with transplanted pituitary tumor, too few animals were involved to provide significant data. In general the proximal tibial epiphysis was wider on the left (adjacent to tumor) than on the right, and the left epiphysis in experimental animals was wider than that of unoperated controls. Tumor could not be demonstrated in sections of tissue recovered from the graft site. This was not surprising in view of the long "incubation" period noted on initial transplantation.

DISCUSSION

PART I

The increased width of the left tibial epiphyseal cartilage plate in all mice with pituitary grafts suggests that the pituitary gland is capable of exerting a somatotrophic effect even when anatomically separated from the hypothalamus. The fact that the right (sham-operated) cartilage plate in hypophysectomized mice was not significantly narrower than the control value raises the possibility that the somatotrophic effect may have been systemic as well as local; however, this cannot be stated with certainty because hypophysectomized controls were not included in the study. The decreased axial skeletal growth in hypophysectomized mice argues against a systemic effect although it may be that the cartilage plate is simply a more sensitive receptor.

The intact mice treated with growth hormone did not increase greatly in body weight or skeletal growth considering that 1 mg. per day is a rather massive dose. This may be due to the short experimental period or to the fact that the animals were injected during a phase of rapid growth when endogenous levels of growth hormone are presumably high. Girard, et al. (22) studied serum concentrations of growth hormone in patients of all ages and found that mean values for children were significantly greater than those in adults, with the highest levels obtained in adolescents. Previous studies (23, 24) showing a dramatic skeletal response to somatotrophin utilized older animals that had reached a

growth plateau and injections were continued for more than one year.

The demonstration that pituitary glands transplanted to the epiphyseal cartilage plate exert a somatotrophic effect is only suggestive evidence for autonomous production of growth hormone. The tibia test specifically indicates growth hormone only when a linear dose-response relationship is obtained. Marx, et al. (25) studied the specificity of the tibia test and found that thyroxin and prolactin produced slight increases in width of the cartilage plate. These findings were confirmed by Geschwind and Li (16) although with their prolactin preparation the response was much more striking in males than in females. Scooley, et al. (26) administered prolactin as replacement therapy for hypophysectomized pigeons and found that treated birds had an increase in body weight and visceromegaly compared to controls. This effect of prolactin is particularly relevant in view of the evidence which has been presented showing an increased output of lactogenic hormone when pituitary glands are transplanted to extrasellar sites in mice (27) and when the pituitary is separated from the hypothalamus as in surgical stalk section in man (28, 29). Interruption of the hypothalamo-hypophyseal system with increased prolactin secretion may also be a factor in the etiology of galactorrhea seen in the Chiari-Frommel syndrome (30).

Difficulties in interpretation of the tibia test led to the search for more sensitive and specific immunologic methods.

The technique of Read and Bryan (31) utilizing hemagglutination inhibition has yielded consistent results which have been duplicated in other laboratories. However, the finding of Ehrlich and Randle (32) of elevated levels of growth hormone in patients studied 2-10 days post-partum when lactotion had commenced suggests either that growth hormone may have a role in milk production in women or that the hemagglutination inhibition technique also detects prolactin.

In another method using radio-sulfate incorporation into cartilage in vivo (33) and in vitro (34) as an index of growth hormone activity, costal cartilage responded significantly to both prolactin and STH.

Recently a radio-immunoassay has been developed by Utiger, et al. (35) which depends on the ability of unlabeled growth hormone in plasma samples to competitively inhibit the binding of I^{131}- labeled growth hormone by STH antibodies. A variant of this assay devised by Greenspan, et al. (36) uses I^{131}- labeled growth hormone antibody. The existence of nonspecific antigens in plasma has been a major obstacle to the application of both methods.

In view of the impressive biological overlap between growth hormone and prolactin, and the difficulty encountered in precise measurement of either hormone, the present experiments do not furnish a clear cut answer to the question of autonomous production of growth hormone by the pituitary gland. While normally the hypothalamus probably acts as a regulator of STH synthesis and release, it appears that the

isolated pituitary can continue to secrete in suboptimal amounts a hormone or hormones with somatotrophic properties.

PART II

The conclusion that the pituitary tumor studied exerted a primarily somatotrophic effect is based on the following evidence: (1) tumor-bearing animals were substantially larger than controls in terms of body weight and skeletal development although they did not appear obese or edematous, (2) the body weight difference between control and experimental animals was far greater than could be accounted for on the basis of tumor weight alone, (3) relative weights of liver, spleen, and kidneys were significantly greater in mice with tumors, and (4) there was no indication of thyroid, adrenal, or gonadal stimulation in mice of either group.

After the tumor had reached sufficient size for its host to be included in the experimental group (greater than 5 x 5 mm.) no relationship was noted between tumor size, latency period, or duration and extent of somatotrophic effect.

The control group in the present study was composed of mice with tumor transplants that did not "take". This differs from the type of control used in previous experiments dealing with pituitary tumors in which non-grafted animals matched as to sex and age were employed. The reasons for failure of a tumor transplant to "take" are poorly understood. Aside from technical errors such as bacterial contamination of the graft, Furth, et al. (37) postulated that modifications

in tumor cells may occur during transplantations such that cells are no longer fully autonomous or histocompatible. The present tumor was in its fourth transfer generation. However, portions of the same tumor were transplanted to genetically identical hosts and in the majority of animals the tumor grew. It is highly unlikely that in the arbitrary subdivision of a tumor mass some animals would have been given only incompatible or dependent cells.

It is conceivable that the tumors had a selective influence and tended to flourish in the largest, healthiest animals. It is a popular clinical impression that neoplasms grow more rapidly in young, vigorous patients and that cases of extremely slow progression occur in aged patients. As Homburger (38) has pointed out, however, "Careful scrutiny and statistical evaluation indicate that the course and natural behavior of most cancers is approximately the same regardless of age."

The control and tumor-bearing mice were comparable in weight at the beginning of the experiment and all mice were approximately the same age at the time of tumor transplantation. Mice in the control group were on the average 150 days older at autopsy than mice in the tumor-bearing group. The weight curve for control mice reached a plateau at about 13 months and remained stable for the rest of the experiment. It is doubtful, therefore, that the reduced weight of control animals could be attributed to some form of senile cachexia.

Furth, et al. (37, 39) using total-body irradiation and large doses of estrogen induced pituitary tumors with mammo-somatotrophic features in mice and rats. Host animals were much heavier than non-grafted controls and had enlarged livers, spleens, and kidneys. In addition, marked alveolar hyperplasia resembling that of pregnancy was present in the mammary glands of females. (In the present experiment mammary glands were not studied in detail; however it was noted at autopsy that 3 out of 5 tumor-bearing females had well developed mammary glands with milk-filled ducts, whereas the mammary glands of control females showed little evidence of activity.)

Although in general there was lack of peripheral endocrine organ stimulation with these tumors, the thyroid was moderately enlarged in some animals (40).

Kim, et al. (41) described spontaneous chromophobe adenomas in rats with marked mammo-somatotrophic effects. These tumors resembled those induced by irradiation or estrogen both in histologic appearance and biological activity.

Schlumberger (42) reported a somatotrophic pituitary tumor in parakeets which resulted in obesity, hyperglycemia, and enlarged livers with heavy accumulation of fat in hepatic cells. The tumor showed very little ACTH activity as determined by the adrenal ascorbic acid depletion test, no prolactin by the pigeon crop gland assay, and a low level of STH activity as measured by the tibia test. While the tumor may have been secreting growth hormone, the presence of obesity

and fatty infiltration of the liver suggests that ACTH was the major product despite the appearance of normal adrenals and an equivocal ascorbic acid test.

The biological overlap between prolactin and growth hormone was mentioned earlier as a factor complicating interpretation of the tibia test. Problems are also encountered in evaluating reports of mammo-somatotrophic pituitary tumors because several investigators have recently been impressed by the luteotrophic properties of growth hormone.

Ferguson and Wallace (43) found that 3 preparations of human growth hormone examined by zone-electrophoresis on starch-gel showed a striking similarity to sheep prolactin in the mobility and relative amounts of principal components. Biologic assay of sheep prolactin and Raben human growth hormone (44) for crop-sac stimulating activity gave relative potencies of the same order. These authors felt that the luteotrophic activity of STH was not due to contamination but was a function of the same molecules that possess growth hormone activity.

Chadwick, et al. (45) assayed human pituitary fractions for STH activity by the tibia test. Their fraction 4 which was markedly somatotrophic also showed high lactogenic potency when administered in intraductal injections into the mammary glands of pseudopregnant rabbits. Contrary to the results of Ferguson and Wallace (43) this fraction had no measurable crop-stimulating activity in the pigeon.

Kovačić (46, 47) has shown that human growth hormone has luteotrophic activity similar to bovine prolactin both in prolonging diestrus in the mouse and in inducing deciduoma formation in damaged uterine horns or hypophysectomized mice.

Clinically the incidence of lactorrhea in women with acromegaly has been estimated to be 4% (48) and even gynecomastia has been reported in this syndrome (49, 50).

In none of the previously cited tumor studies was skeletal data presented to document the somatotrophic effect claimed although experimental and clinical work with growth hormone has concentrated mainly on the skeletal system. Freud, et al. (51) studied the effects of hypophysectomy and maintenance with growth hormone on the development of vertebrae, ribs, and tibia of the rat. They were attempting to localize the point of attack of STH and considered tail length and length of individual caudal vertebrae valuable valuable indicators of growth hormone activity.

Asling, et al. (24) carried out long term studies with growth hormone in hypophysectomized and unoperated adult rats. Gigantism was induced in these animals with increased body length largely attributable to vertebral growth. Changes in the vertebral column seen during life of the animal included limitation of movement, curvatures, and fixation in the direction of kyphosis especially in the lumbar region. The finding of active vertebral epiphyseal plates was thought to account for the increased length of the vertebral column. These

observations correlate well with the increased length of lumbar, sacral, and caudal vertebral segments obtained in the present experiment.

Marie (52) described hypertrophic skeletal changes in the syndrome he named acromegaly in 1886, thereby providing one of the earliest clinical models for studies in experimental gigantism. Changes in the axial skeleton of acromegalics were recognized by Cushing and Davidoff (53) and carefully analyzed by Erdheim (54). Erdheim described overgrowth of the ventral and lateral surfaces of vertebral bodies especially at the disc margins. The bony increments were thought to develop from the periosteum and by endochondral ossification of proliferating cartilage. Similar vertebral changes have been reported by Waine, et al. (50) and Kellgren, et al. (55); both groups have stressed the significance of acromegalic arthropathy as a clinical entity. While no pathologic changes were detected in the axial skeleton of tumor-bearing animals in the present study, bony increments on the ventral and lateral surfaces of the vertebral bodies as described by Erdheim could well account for the marked increase noted in vertebral segment length.

SUMMARY

1. Pituitary glands were transplanted to the left proximal tibial epiphysis in untreated and hypophysectomized mice. A somatotrophic effect manifested by an increase in width of the left epiphyseal cartilage plate was seen in mice of both groups. Treatment of intact mice with bovine growth hormone resulted in a similar increase in width of the cartilage plate.

2. Transplanted pituitary glands did not enable growth of the axial skeleton to proceed normally in hypophysectomized mice.

3. A pituitary tumor with somatotrophic properties is described. Mice bearing subcutaneous transplants of this tumor were significantly larger in terms of body weight, organ weights, and skeletal development than control animals with little or no tumor.

4. The possibility that these somatotrophic effects were due to autonomous production of growth hormone by the transplanted pituitary and the pituitary tumor is discussed.

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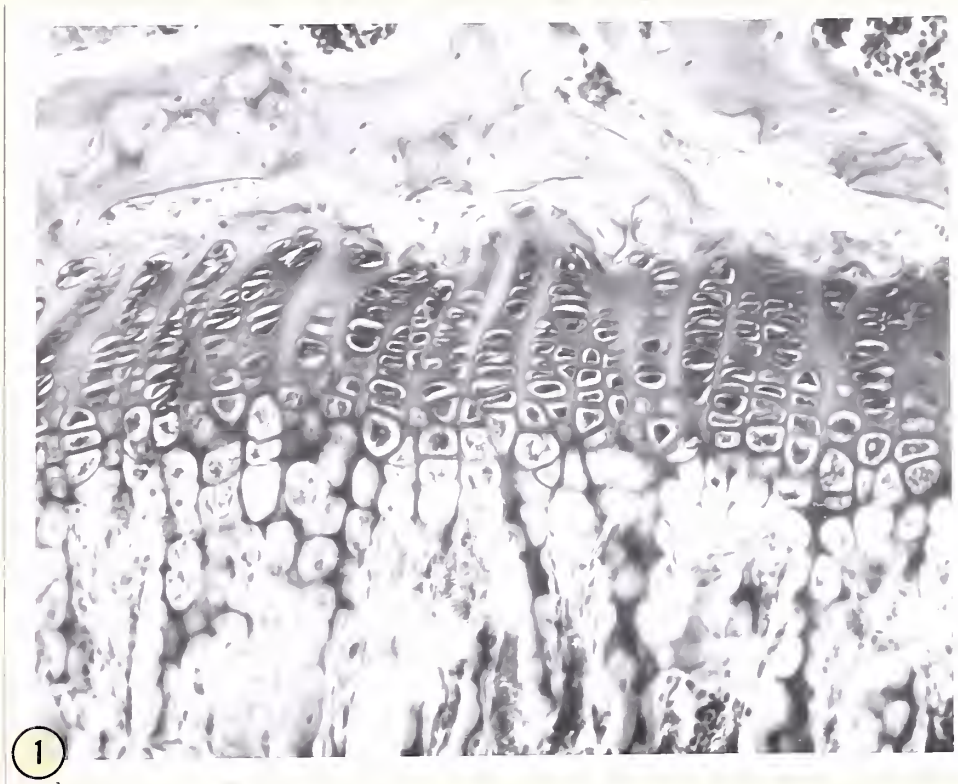
Figure 1. Left tibial epiphysis from an untreated mouse. Cartilage plate measured 242.1 microns. (x400)

Figure 2. Left tibial epiphysis from a mouse treated with bovine growth hormone for 3 weeks. Cartilage plate measured 310.6 microns. (x400)



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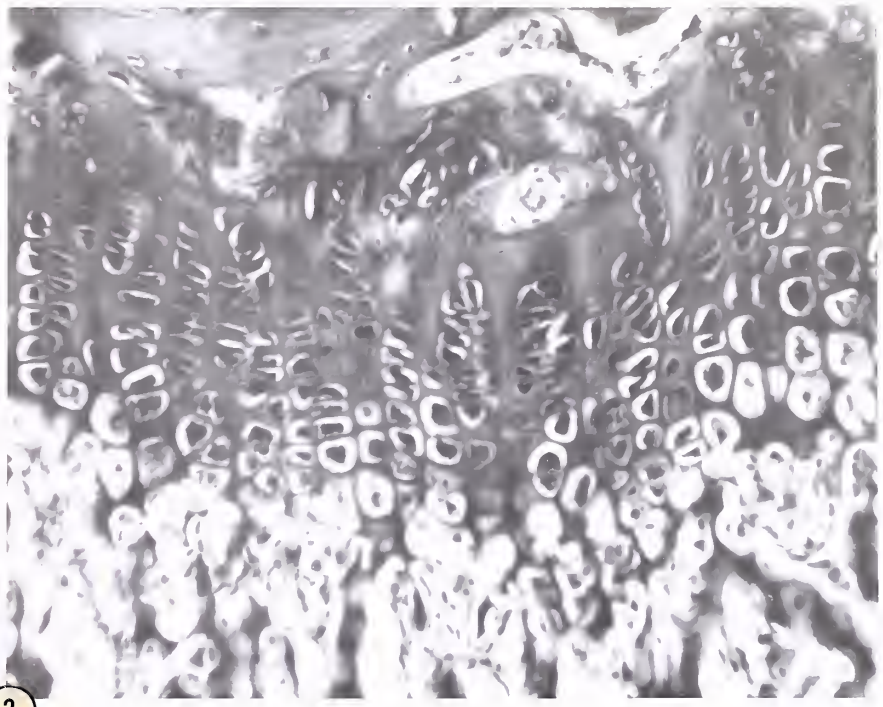

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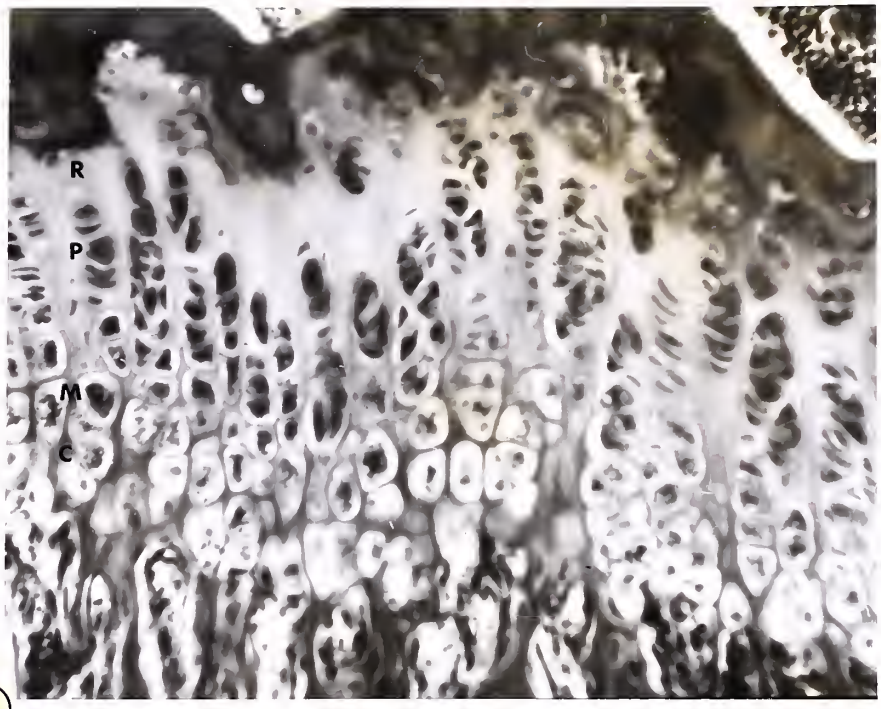
Figure 3. Left tibial epiphysis from a pituitary-transplanted mouse showing zones of resting (R), proliferating (P), maturing (M), and calcifying (C) cartilage. Cartilage plate measured 322.2 microns. (x400)

Figure 4. Right tibial epiphysis from same mouse as in Fig. 3. Cartilage plate measured 260.8 microns. (x400



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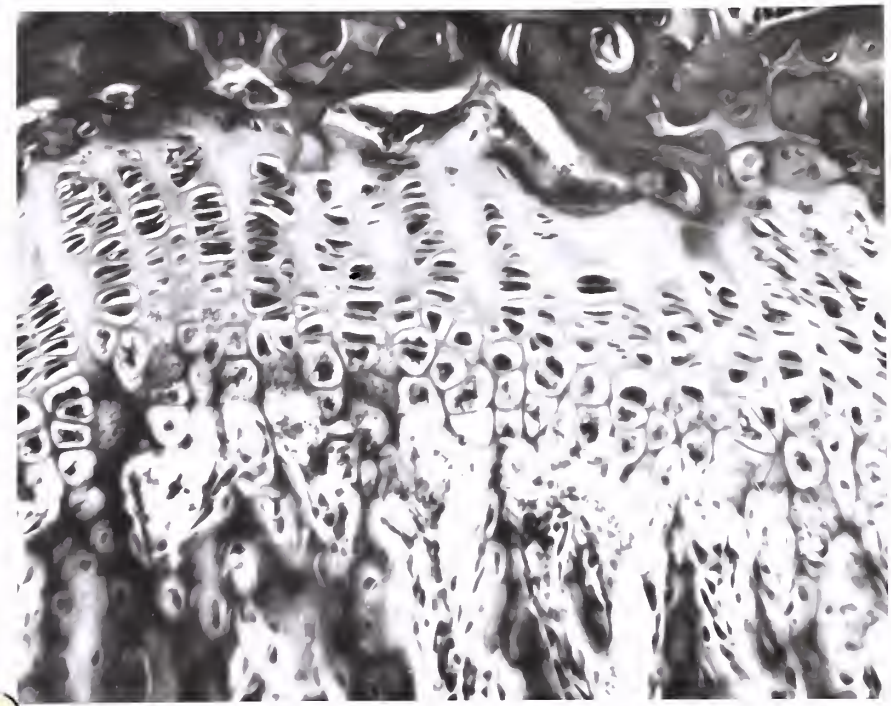

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Figure 5. Left tibial epiphysis from an hypophysectomized mouse with pituitary transplant. Cartilage plate measured 348.0 microns. (x400)

Figure 6. Right tibial epiphysis from same mouse as in Figure 5. Cartilage plate measured 260.8 microns. (x400)

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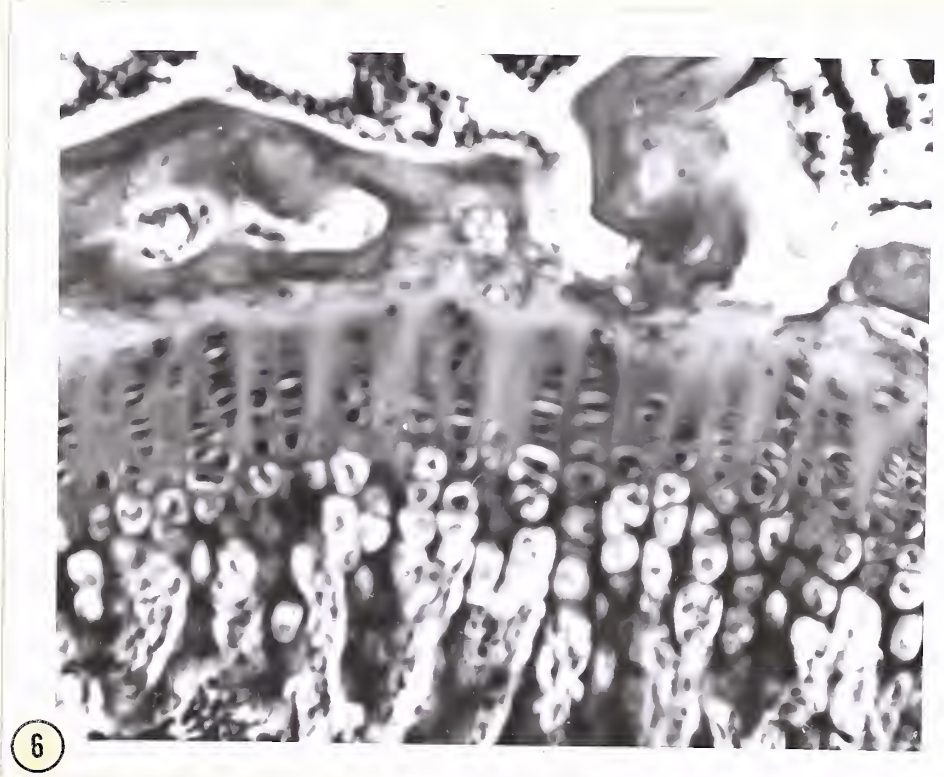

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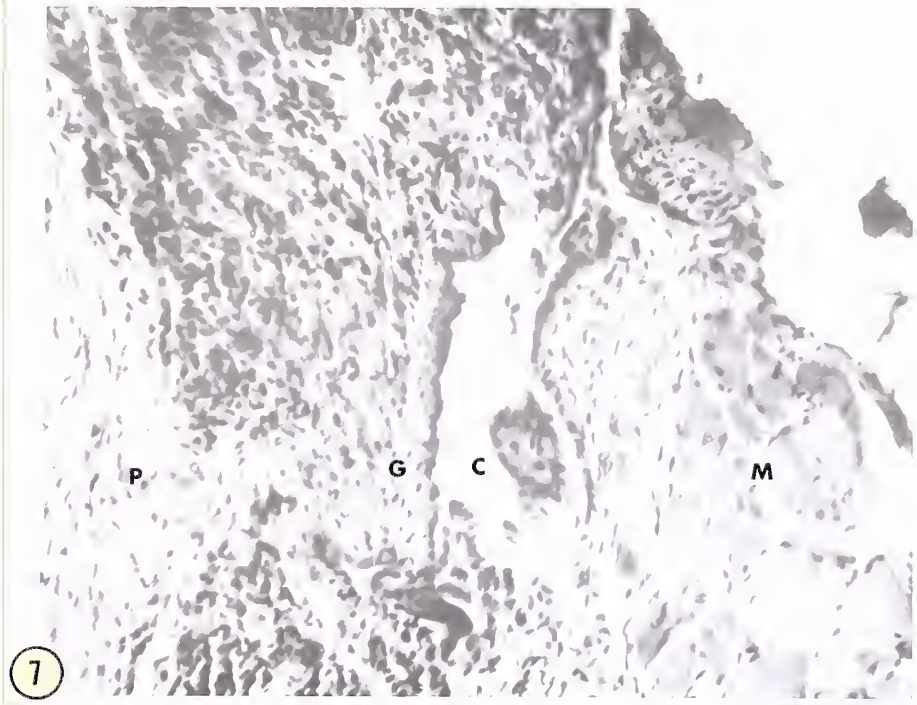
Figure 7. Section of tissue recovered from graft site in pituitary-transplanted mouse. Fragments of pituitary graft (G) surrounding residual cleft (C) are wedged between periosteum (P) and striated muscle (M). (x400)

Figure 8. Section of tissue recovered from graft site in pituitary-transplanted mouse showing fragments of pituitary graft (G) surrounding residual cleft (C). (x400)



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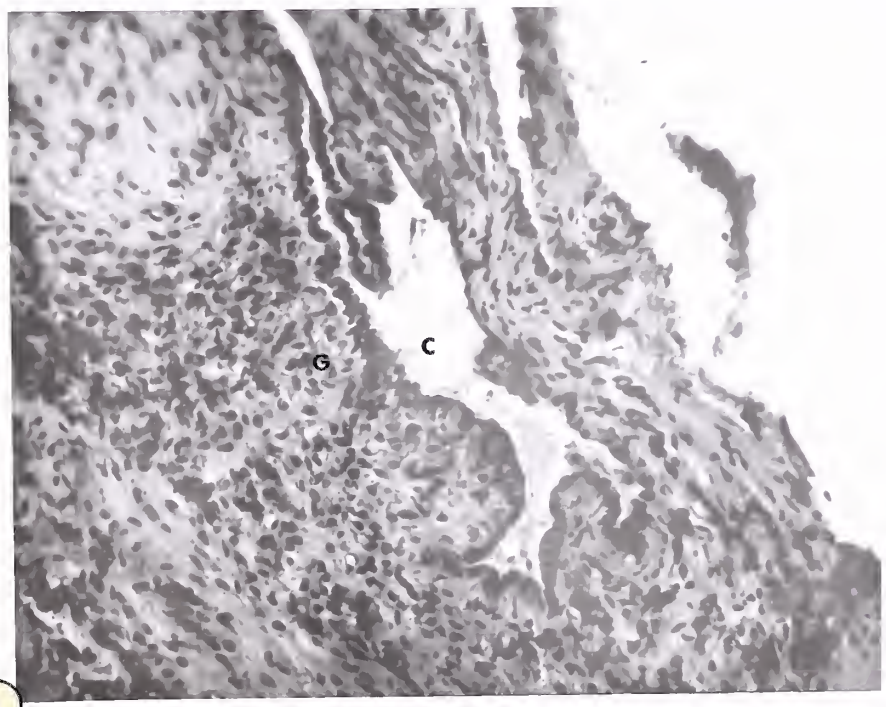

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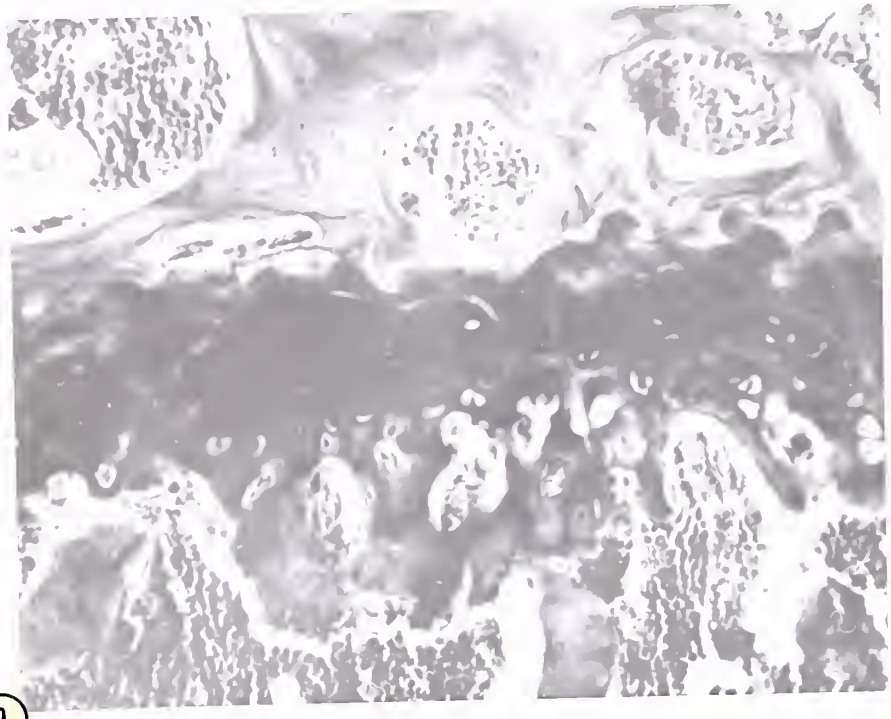
Figure 9. Left tibial epiphysis from tumor-bearing mouse 412 days of age at autopsy. The cartilage plate is sealed by bone on both epiphyseal and diaphyseal surfaces. (x400)

Figure 10. Histologic appearance of somatotrophic pituitary tumor. Tumor cells fairly uniform in size and shape with moderate amount of cytoplasm; cytoplasmic boundaries generally indistinct.



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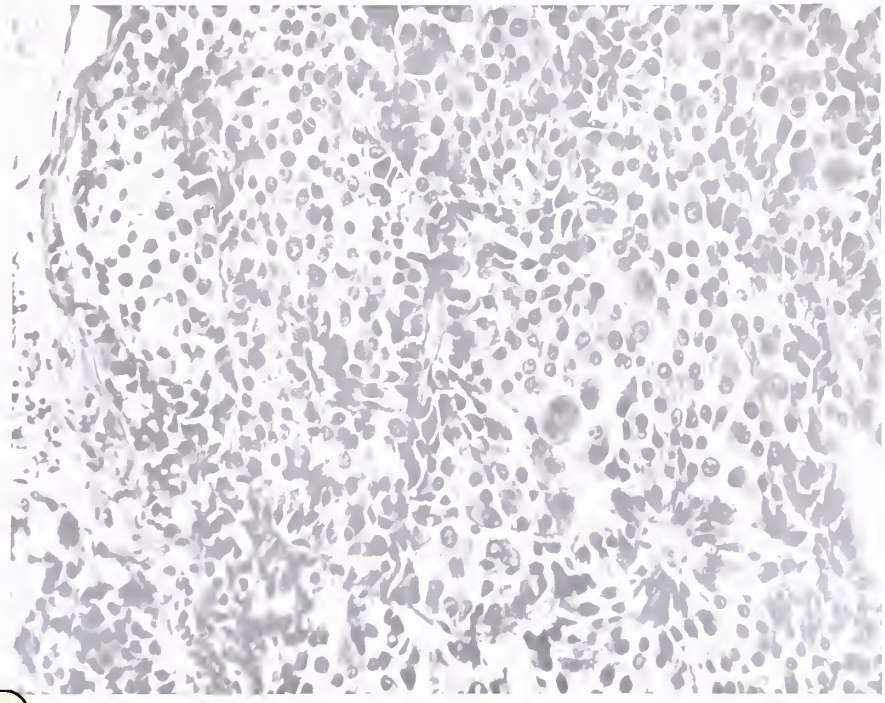

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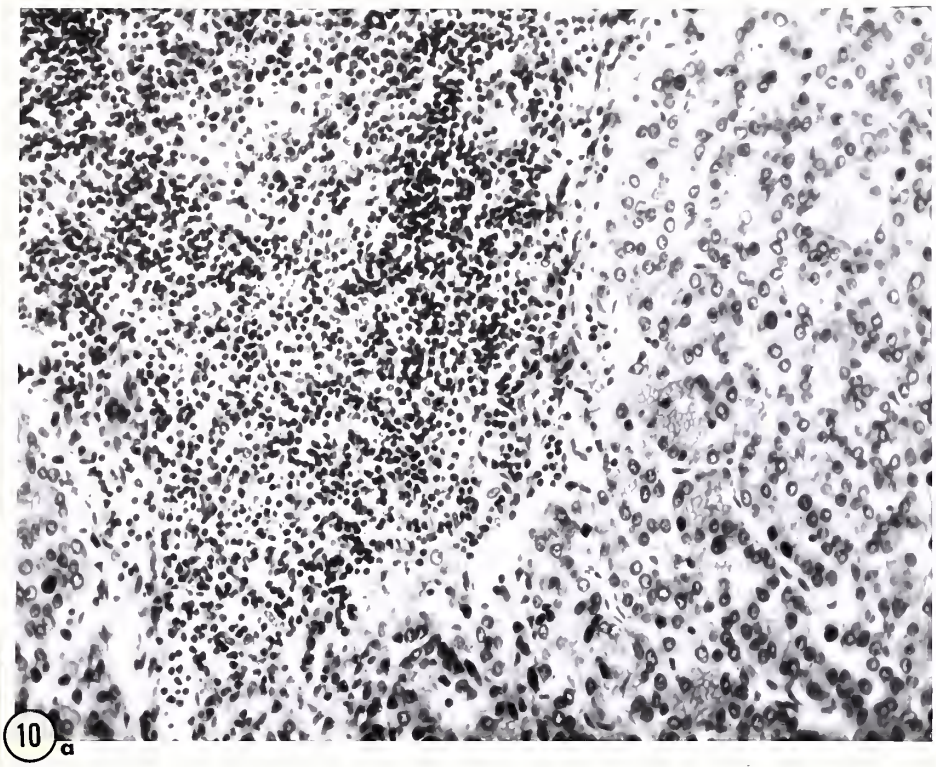
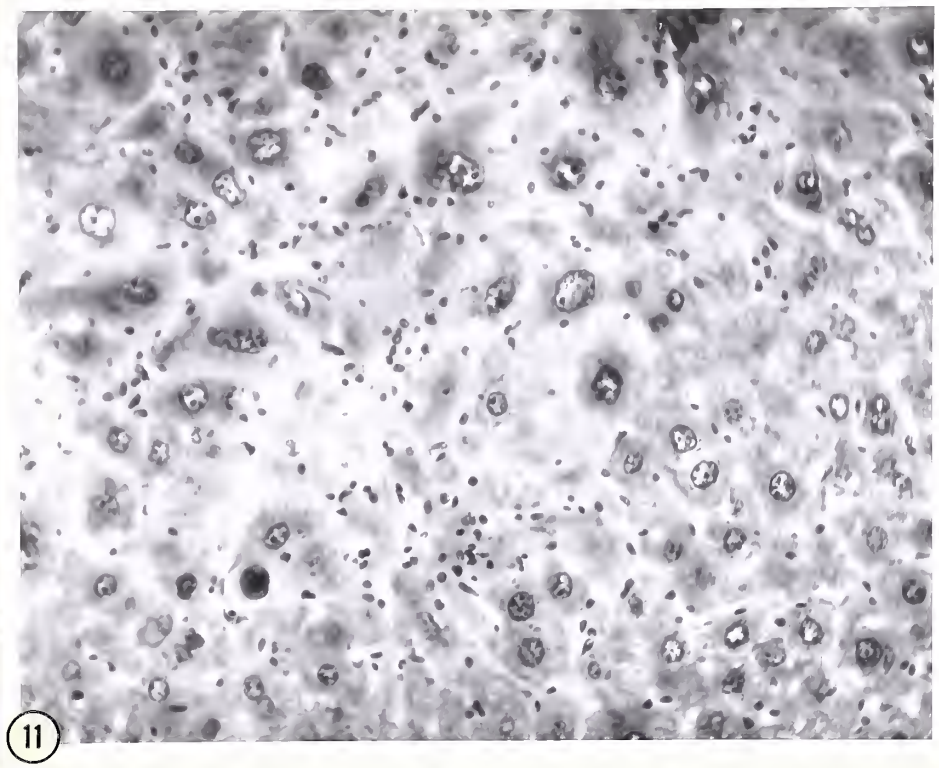
Figure 10a. Section of pituitary tumor showing involvement of adjacent lymph node. Tumor cells are arranged in duct-like structures within which are clusters of erythrocytes. (x400)



10 a

Figure 11. Section of liver from tumor-bearing mouse showing tremendous hypertrophy of hepatic cells with large nuclei and almost complete obliteration of sinusoidal spaces. (x400)

Figure 12. Section of liver from control mouse in tumor series showing normal hepatic architecture with cords of liver cells radiating outward from central vein and preservation of sinusoids. (x400)



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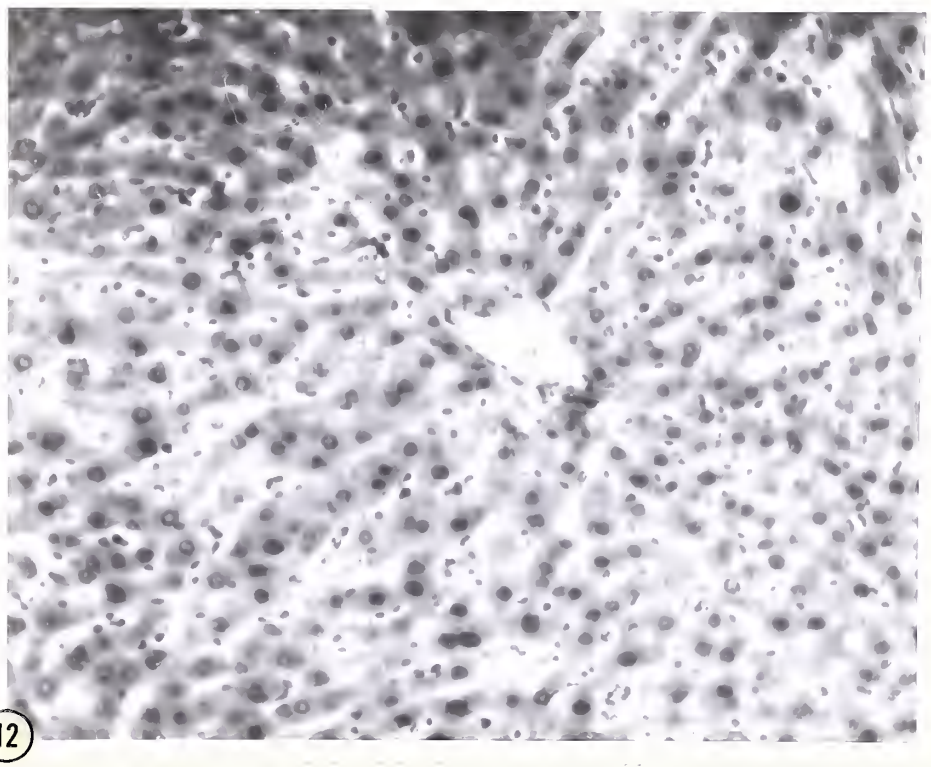

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Figure 13. Skeletal roentgenogram of an untreated mouse. Arrows demarcate lumbar, sacral, and caudal vertebral segments as measured in experiment. Tibial epiphyses are open. (x1.4)

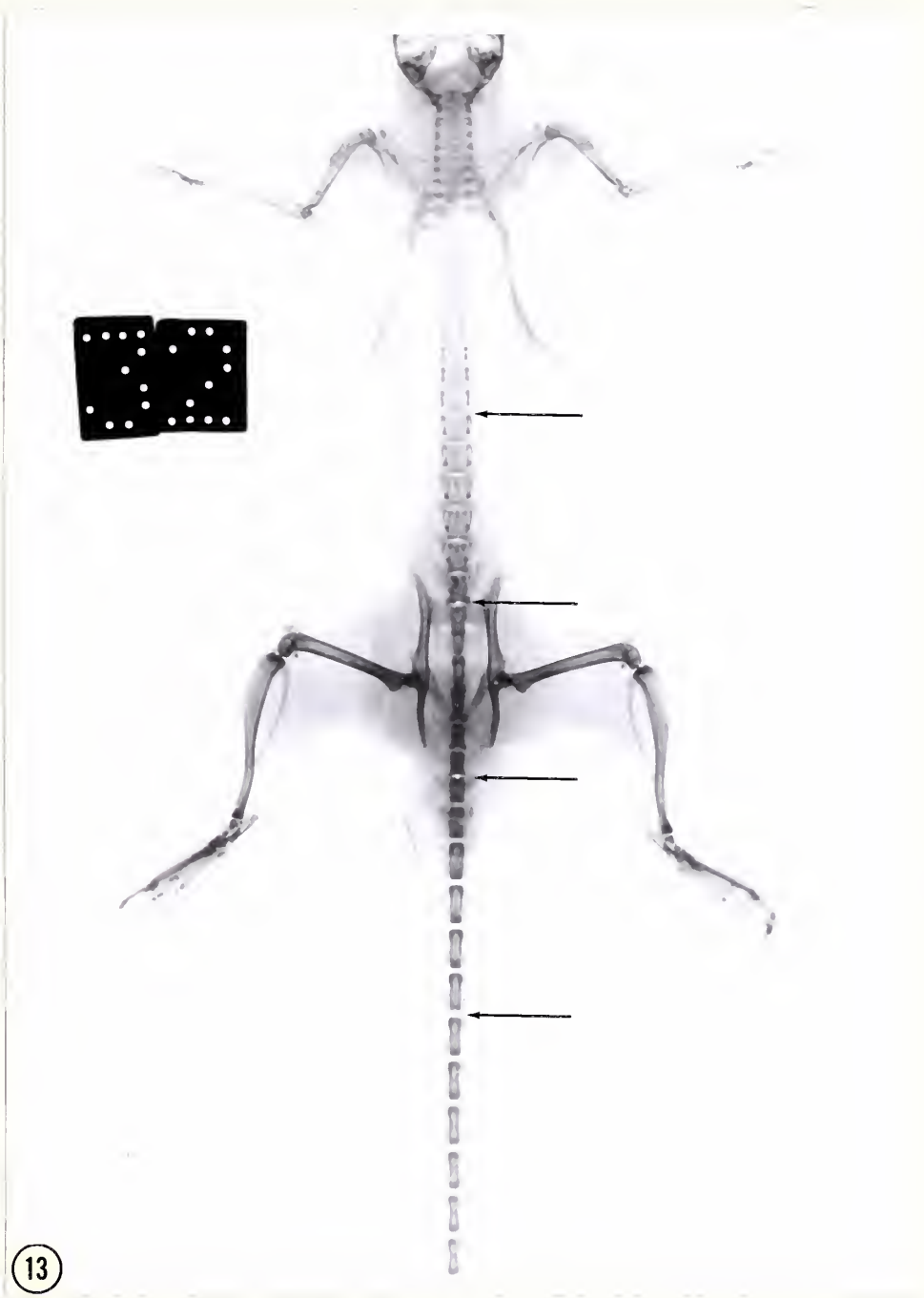

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Figure 14. Skeletal roentgenogram of an hypophysectomized mouse with 2 pituitary glands transplanted to the left tibial epiphysis. Tibial epiphyses are open. (x1.4)

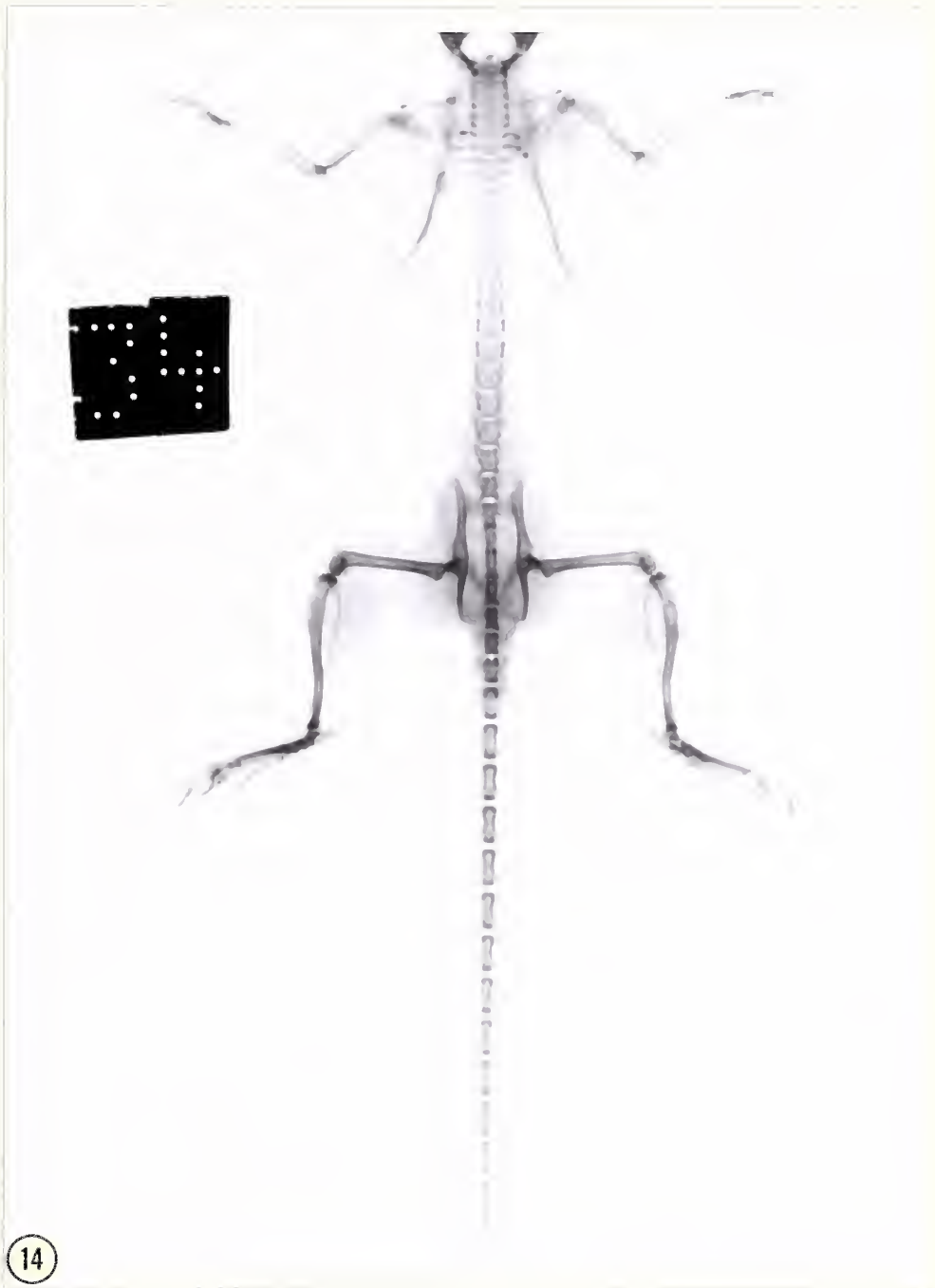

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Figure 15. Skeletal roentgenogram of a mouse treated with bovine growth hormone for 3 weeks. Tibial epiphyses are open. (x1.4)

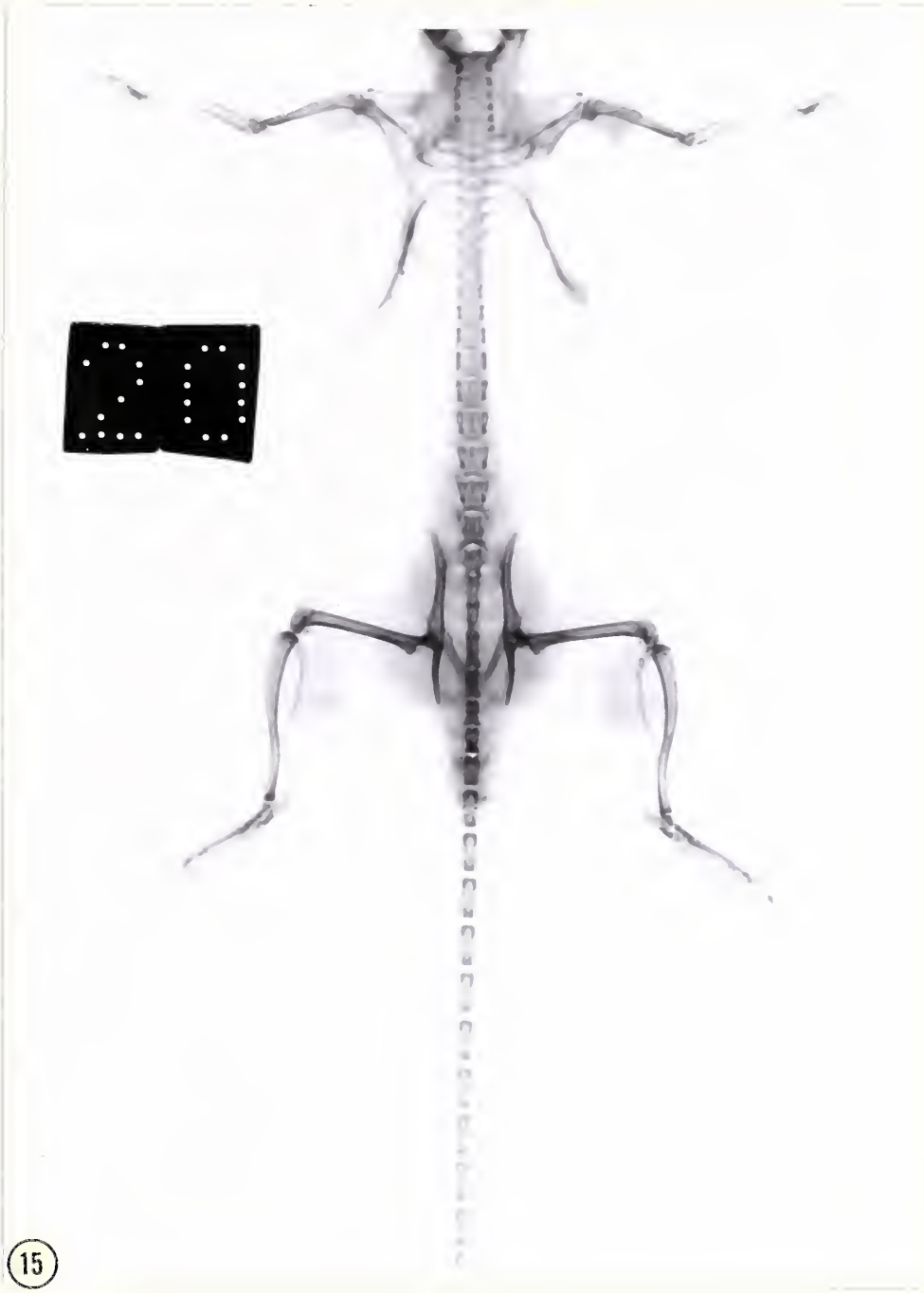

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Figure 16. Skeletal roentgenogram of a tumor-bearing mouse 412 days of age at autopsy. Tibial epiphyses are closed. (x1.4)

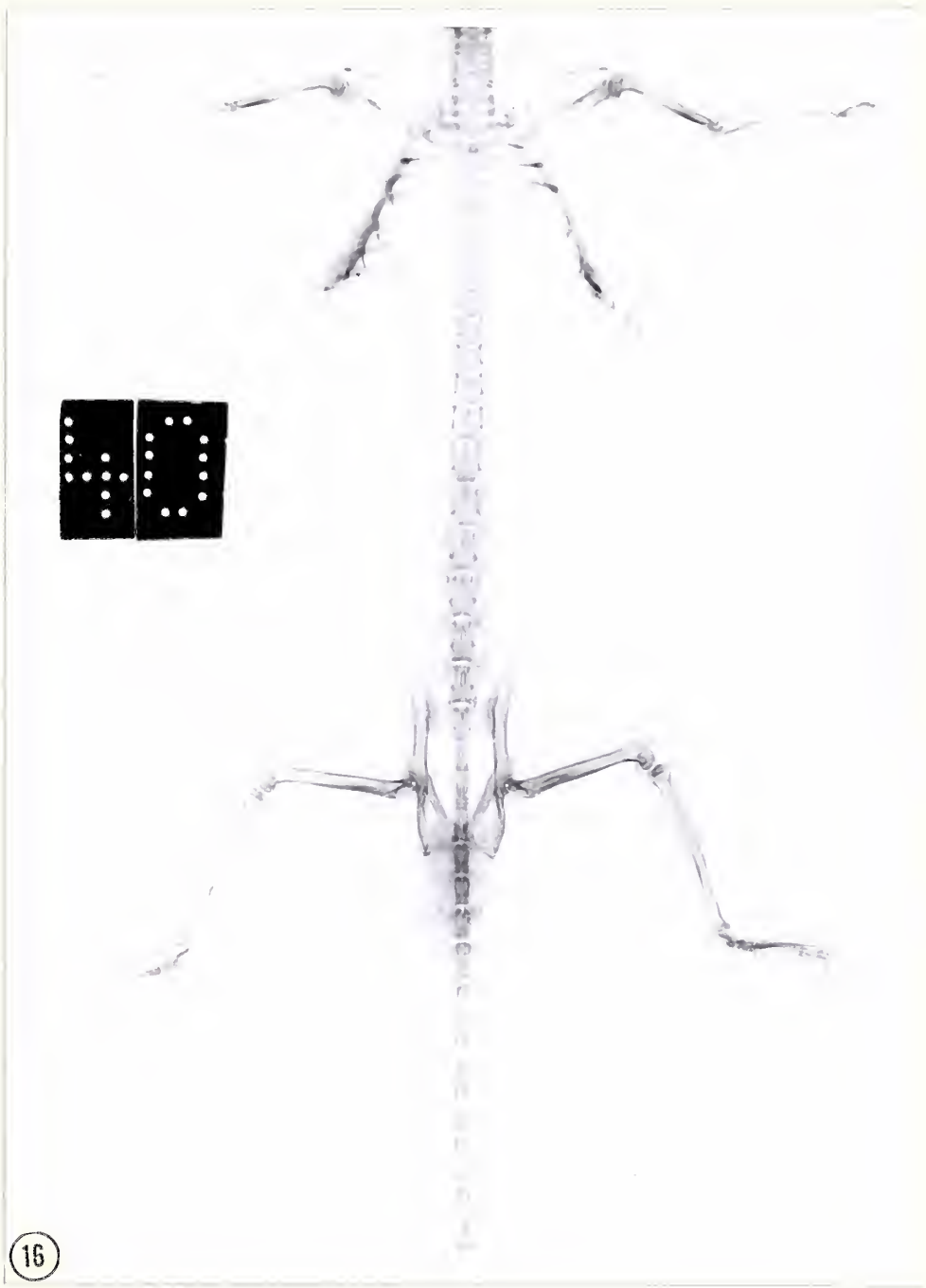

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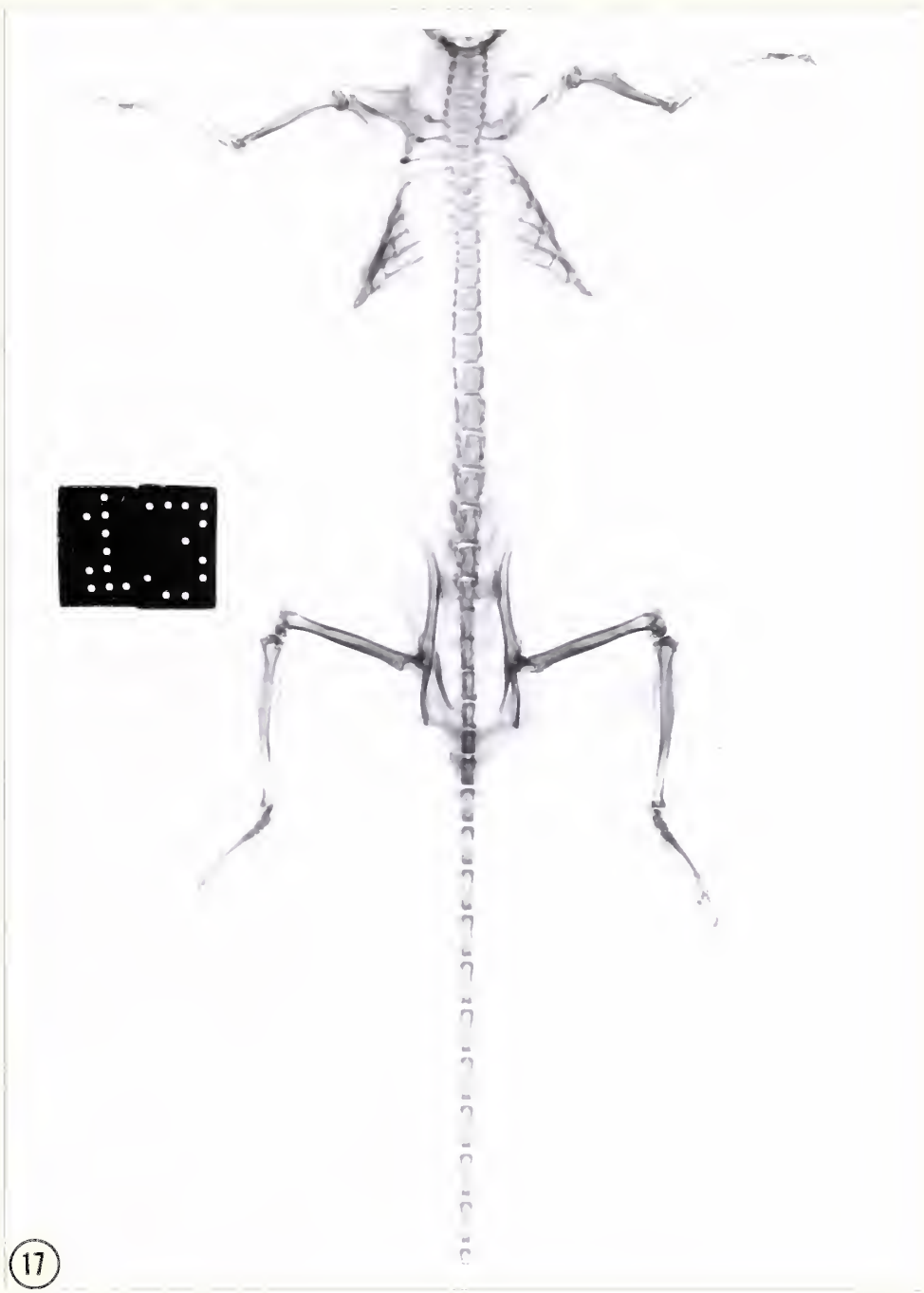
Figure 17. Skeletal roentgenogram of a control mouse in tumor series 670 days of age at autopsy. Tibial epiphyses are closed. (x1.4)



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